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UNPUBLISHED PRELIMINARY DATA

Research on Detection of Extraterrestrial
Life by Ultraviolet Spectrophotometry

Final Report

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Office of Grants and Research Contracts Codes
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16109   Abstract

A research program was carried out to determine the feasibility of applying absorption of far ultraviolet radiation by peptides to the detection of extraterrestrial life on Mars.

Experiments were carried out on a variety of amino acids, dipeptides, tripeptides, polypeptides, and proteins. It was found that all substances containing peptide bonds showed an absorption maximum in the 185-190 mμ region. A suspension of staphylococcus aureus and extracts of local soil and sand also showed an absorption bond in this region.

Experiments with substances that might give false positive absorptions showed that many nonpeptides similarly absorb in this region. However, it was observed that hydrolysis of the peptides resulted in a decrease in absorbancy, as did hydrolysis of the extracts of soil and sand. This effect allows for the distinction between peptides and nonpeptides.

The effect of pH was studied, and it was found that the carboxyl ion absorbed in this same far ultraviolet region. Acidification to a pH well below the pK of a carboxylic acid resulted in the elimination of this absorption. Such acidification permits the further distinction between aliphatic amino acids and peptides.

It was concluded that it is feasible to apply these results to the detection of extraterrestrial life on Mars. Design constraints for the instrument were derived from the experimental results.   *AUTHOR*

1. INTRODUCTION

This is the final report on NASA Contract NASw-571 of a 12-month research program on the absorption of far ultraviolet radiation by peptide bonds. The study was directed at the evaluation of the feasibility of applying this absorption to the detection of extraterrestrial life.

The assumption is made that if life exists on Mars, its essential chemistry is generally similar to that of terrestrial life. It is then reasonable to examine the various characteristics of biological systems and to choose those which are common to all biological systems. Of the various functional systems and chemical components that may be considered, the protein moiety is an obvious candidate. It can be detected on the basis of a variety of properties by many analytical techniques.

The choice of a particular property may be made at various levels of biological organization. Thus, one may choose to detect a protozoan, a bacterium, or a more specialized structural form. On another level of organization, one may choose to detect a functional enzyme system. In the case of proteins as an example of a characteristic chemical component, the work reported here is concerned only with the peptide bond.

It has been reported that the peptide bond absorbs far ultraviolet radiation in the 185-190 mμ region. However, work in this area has been limited by equipment difficulties. The usual quartz optics available in commercial UV spectrophotometrics begin to absorb quite strongly at 200 mμ and are virtually opaque at 180 mμ. The requirement for the exclusion of oxygen imposes another difficulty.

The present availability of suitable optics makes it possible to intensify the study of far ultraviolet spectrophotometry, and this report describes experiments to determine the feasibility of applying this approach to the detection of extraterrestial life and to determine the optimum conditions for reliably detecting the presence of peptide bonds.

2. EXPERIMENTAL

2.1 Instrumental

The work reported herein was started with a Cary model 15 spectro-photometer[4] and was continued after the first quarter on a Beckman model DK-2A for UV recording spectrophotometer. The monochromaters were calibrated against benzene vapor (redistilled over anhydrous calcium sulfate). All experiments in the vacuum ultraviolet region were carried out under conditions of continuous nitrogen purging.

Water was found to be a satisfactory solvent as long as the path length was short. All experiments were therefore carried out in short-path cells. The quartz used in the optics, as well as the cuvettes, was carefully selected for use in the far ultraviolet region.

2.2 Methods

Solutions were prepared in distilled water, phosphate buffers, sulfuric and hydrochloric acids, ammonium hydroxide, and sodium hydroxide.

For studies on the relationship of absorbancy at 1850 A^o to the number of peptide bonds, bovine serum albumin and gelatin were subjected to enzy-matic hydrolysis; in addition, gelatin was also subjected to acid hydrolysis. The enzymatic hydrolyses were carried out by preparing a 2.4×10^{-5} M stock solution of the protein and a 2.4×10^{-6} M stock solution of the enzyme. A series of test tubes were prepared by mixing 5cc aliquots of the protein and enzyme stock solutions, and the contents of each tube were examined spectrophotometrically at periodic intervals. The reference cells in these experiments contained enzyme as well as solvent.

In studying the acid hydrolysis of gelatin, a stock solution of 1 gm gelatin in 100 cc water was prepared and was then divided into 10 cc aliquots per test tube. A 1.0 cc aliquot of 1.0 N HCl was added to each tube, and the tubes were then placed in a bath of boiling water.

In the case of the extracts of soil and sand, aliquots were stirred with solvent (water, sodium hydroxide or hydrochloric acid) for 20 minutes. The supernatant was filtered through a No. 42 filter and then through a millipore filter. Hydrochloric acid was added to the clear filtrate to adjust the pH, and the spectrophotometric absorption was then studied.

2.3 Materials

Spectrophotometric studies were carried out on the following materials:

Bovine serium albumin	DL-alanyl - DL-alamine
Rilonuclease	DL-alanyl - glycine
Phenylalanine	DL-alanyl - DL-leucine
Glycine	DL leucyl-glycyl-glycine
Alanine	DL-alanyl-glygyl-glycine
Tyrosine	Poly-L-lysine
Tryptophane	Poly-L-glutamic acid
Glycyl Glycine	Polyglycine
Benzene	Acetonitrile
Ethanol	Butyl acetate
Diethyl ether	N.N. Dimethyl formanide
Isopropyl ether	Glycerol
Butyl alcohol	Ethylene glycol
Cyclohexane	Isopropanol

Formaldehyde	Acetic acid
Formamide	Petroleum ether
Hydrogen Peroxide (6%)	1, 4 Dioxane
Ethyl acetate	Methanol
Dichloromethane	Carbon tetrachloride
Acetone	Extracts of local sand
Extracts of local soil	Acetic acid
Serine	Gelatin
Leucine	Pepsin (as used for hydrolysis of protein)

3. RESULTS

(Section 3.1 is an abstract of results reported in the first three
quarterly reports. Details may be found in references 4, 5, and 6.)

3.1 Spectra of Biological Material

All the dipeptide, tripeptide, polypeptides, and proteins, that were
studied, exhibited pronounced absorptions maxima in the 185-195mu region
as shown by a typical spectrum in figure 1.[4][5][6] In all cases where
Beer's law plots were prepared, the experimental results conformed closely
to the predictions of the Beer-Lambert law as shown in figures 2, 3, and 4.

It was noted that the absorptivities were affected by pH. Figures 5
and 6 show that absorption of Bovine Serum albumin and alanylalanine are
affected in a similar way as the pH is varied. In both cases, the absorption
increases with pH and passes through a maximum. It may be seen from figure
6 that the change in pH is associated with a shift in absorption maxima as
well as with a change in absorptivity. (These results are typical of those
obtained with other substances.)

An analysis of the absorption by the various substances shows that
the amino acids also absorb the ultraviolet radiation. The absorptivities
of the aliphatic amino acids are reduced to zero when the pH is reduced
to a value of 1.0. However, the aromatic amino acids absorb in the far
ultraviolet even when the pH is reduced as illustrated by the spectrum
for phenylalanine in figure 7.

The peptides all show absorption spectra similar to that of polylysine
shown in figure 1. The proteins often show two distinct maxima; one of
these is the 185-190 mu region, and the other is a 280 mu and is due to

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Figure 1. Absorption Spectra at pH 1-Polysine
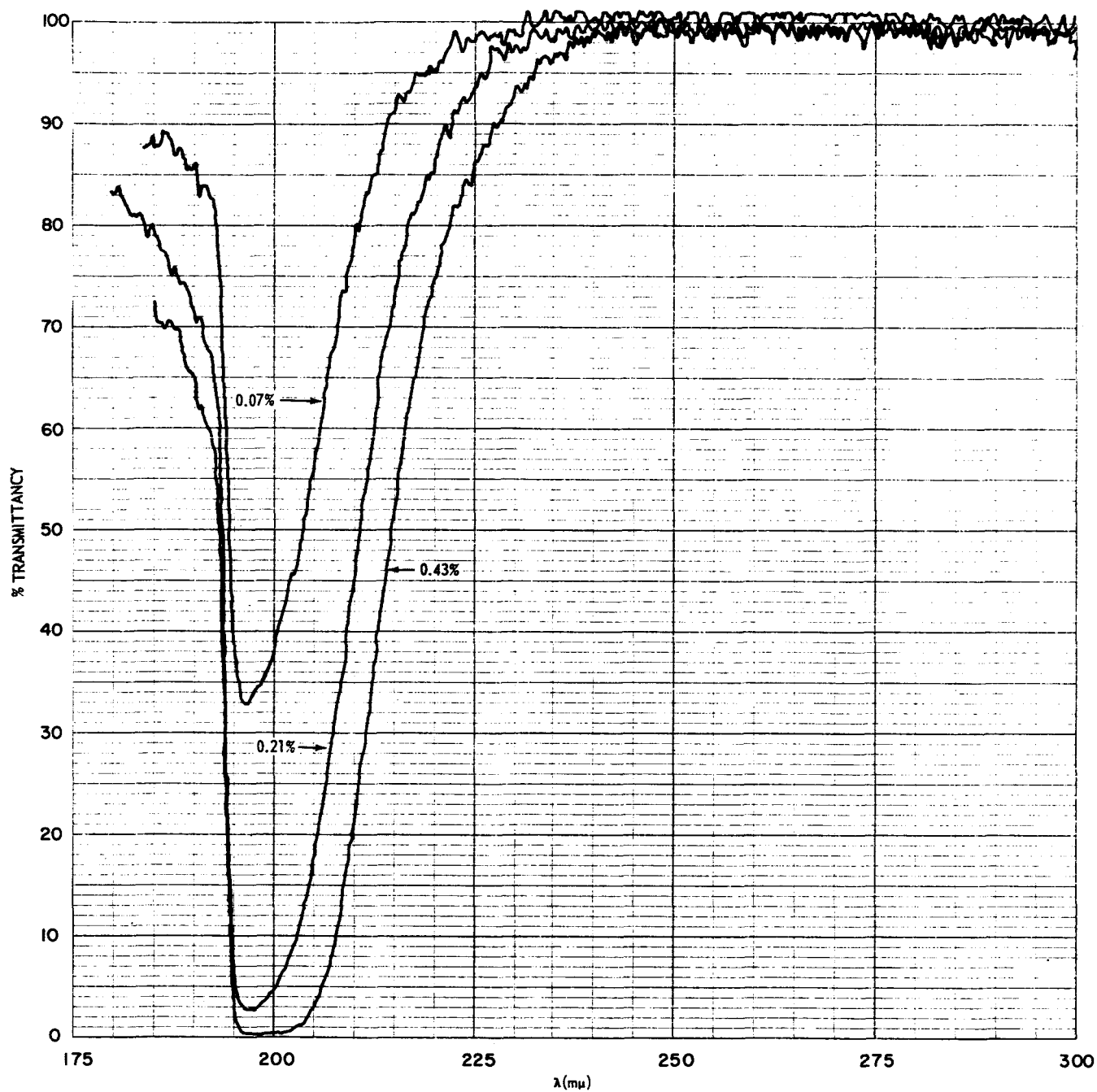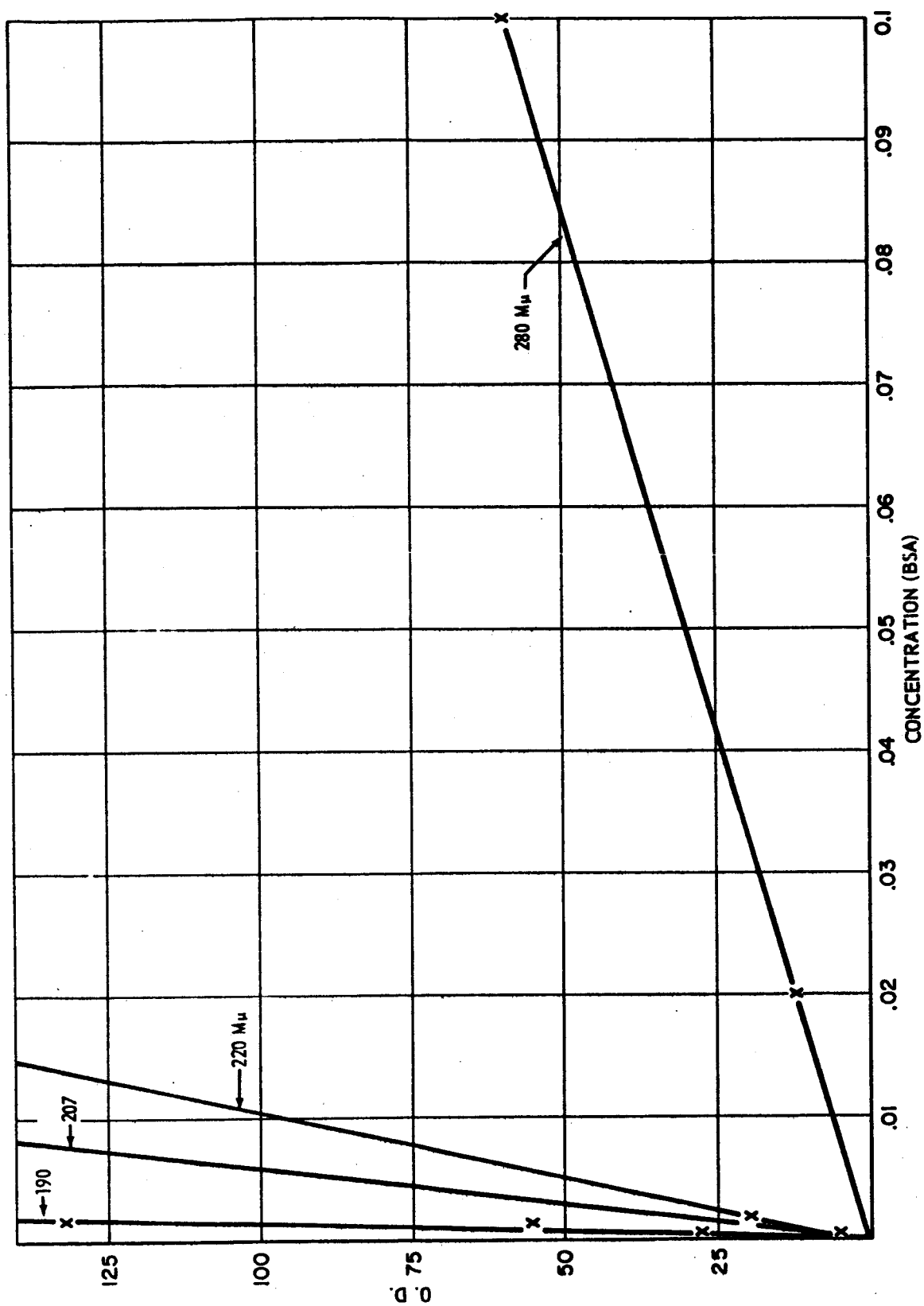
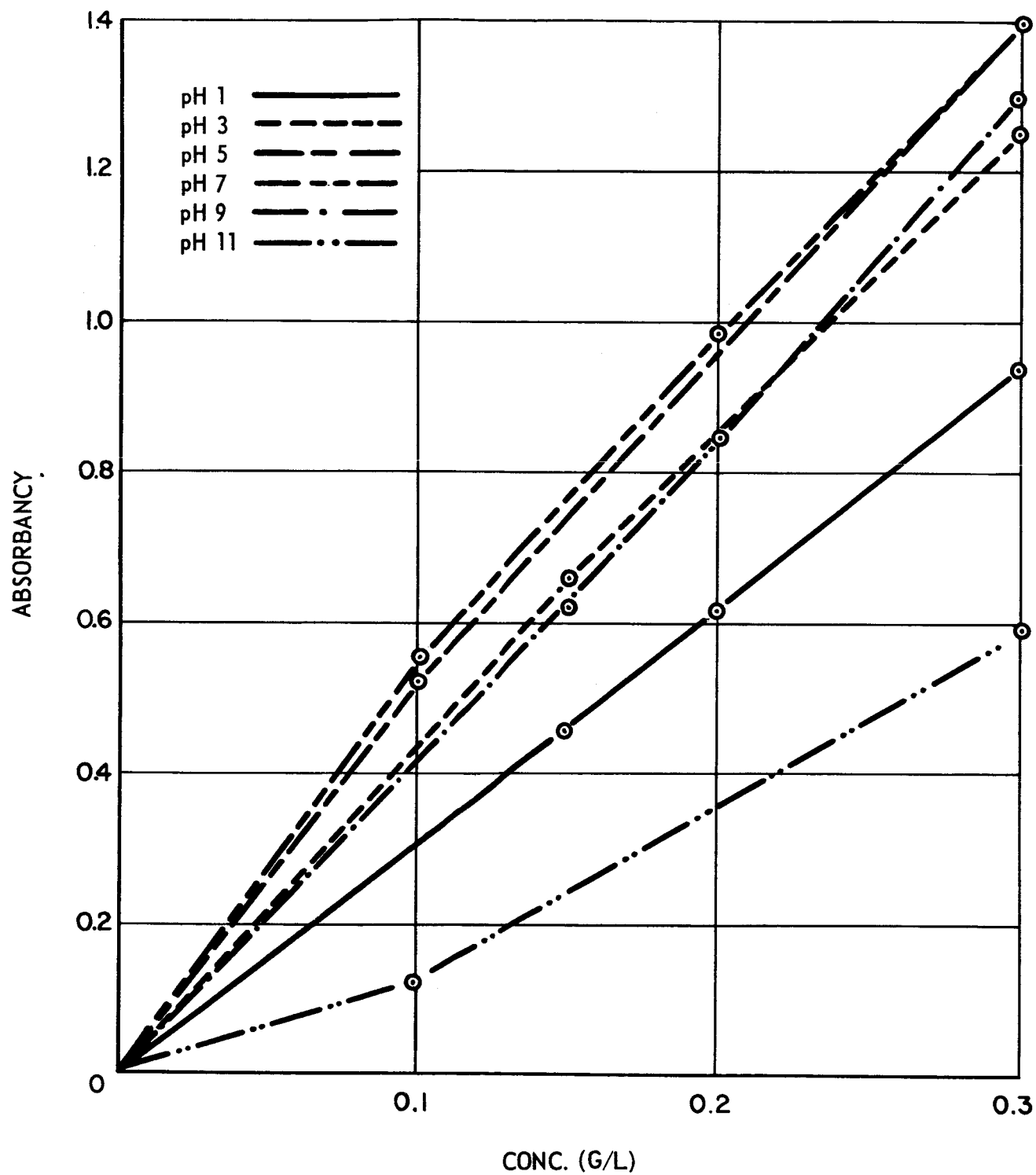Figure 2. Beer-Lambert Plot of Bovine Serum Albumin for Four Absorption Maxima

Figure 3. Beer's Law Plot of Alanylaline at Different pH Factors
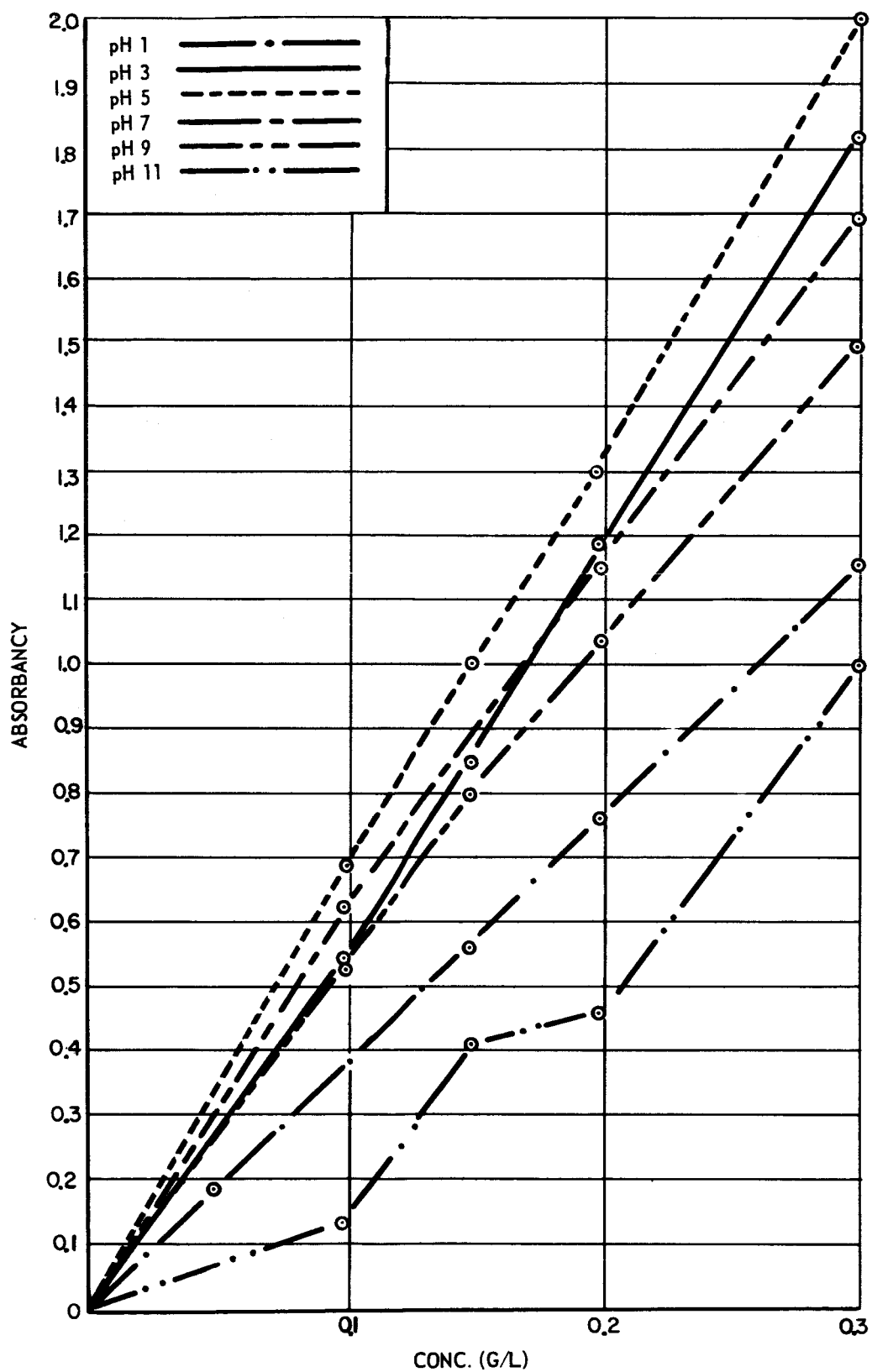
Figure 4. Beer's Law Plot of Alanylglycylglycine at Different pH Factors
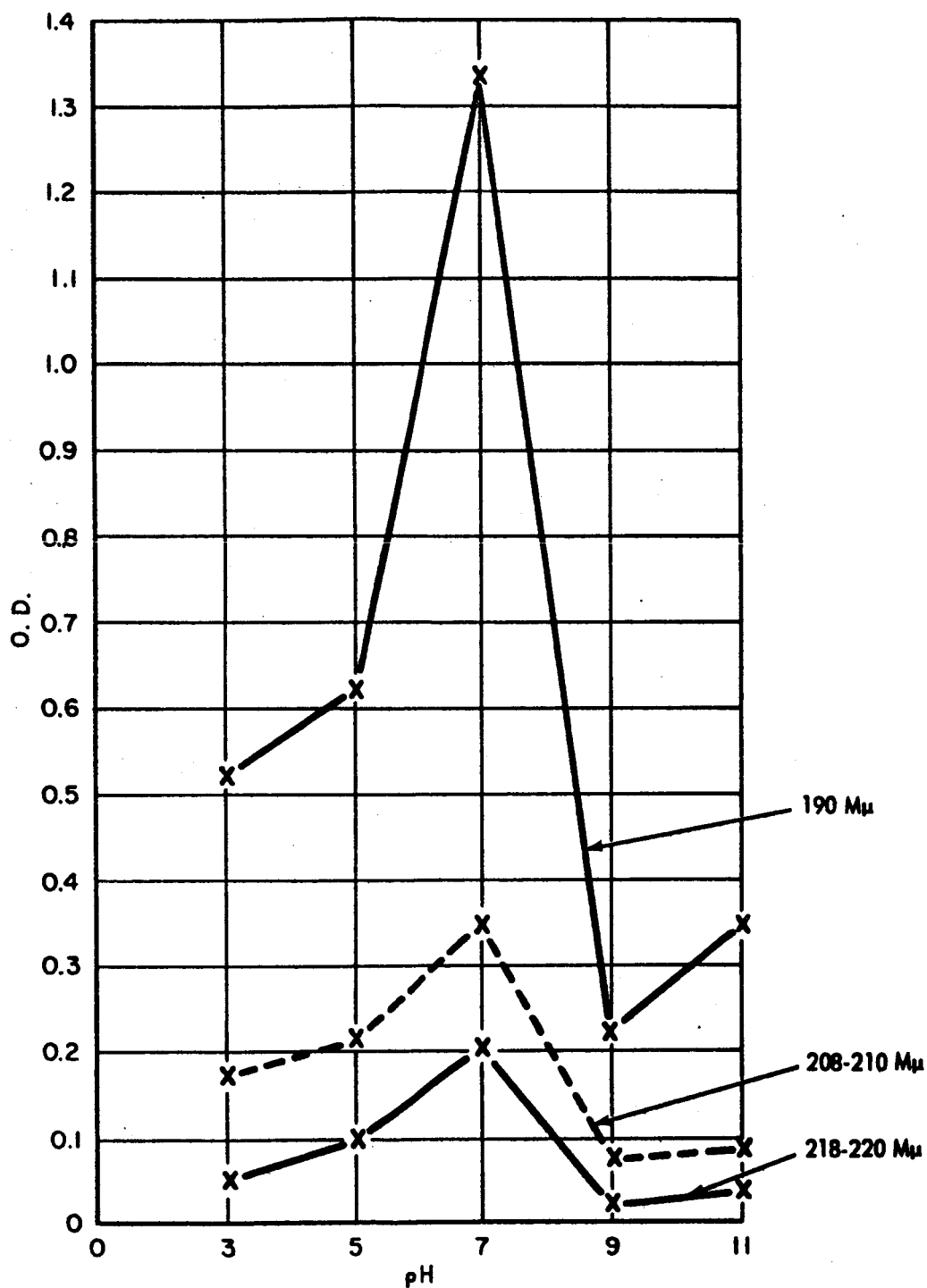
Figure 5. Effect on pH Absorption by Bovine Serum Albumin
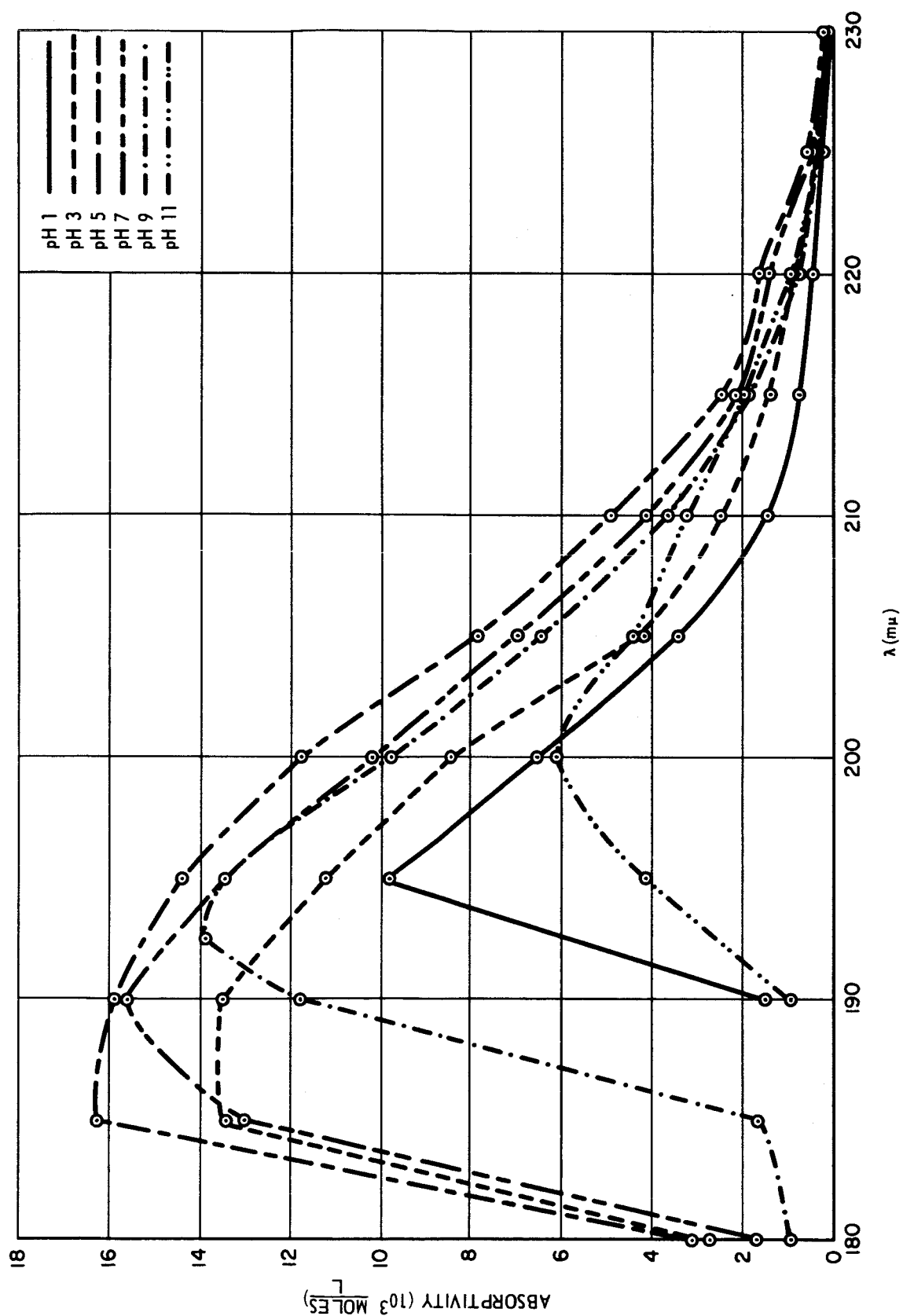
Figure 6. Effect of pH on Absorptivity by Alanylalanine
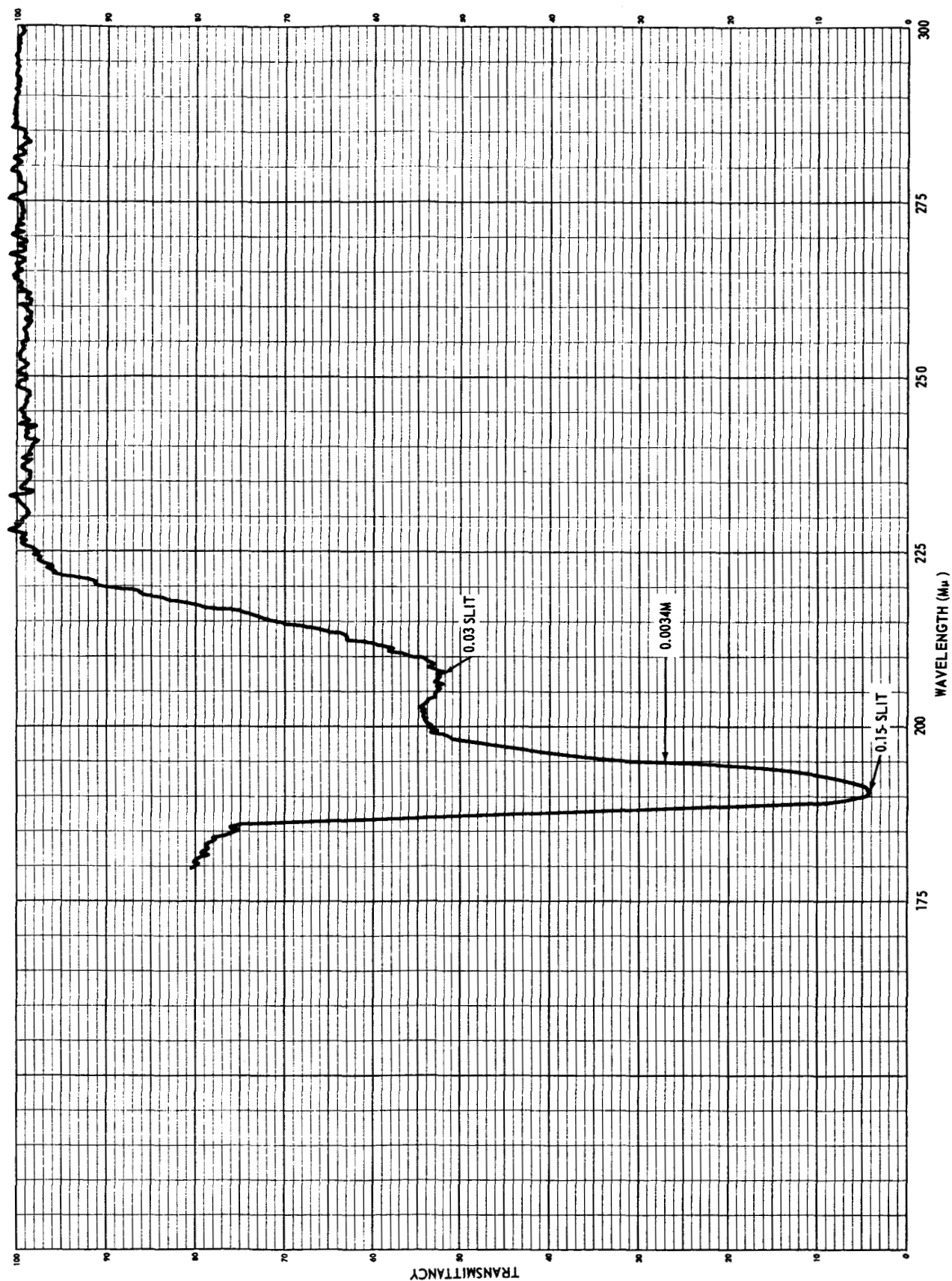
Figure 7. Absorption Spectrum-Phenylalanine

tyrosine. This may be seen in the spectrum shown in figure 8 where the absorption at 280 mu is expanded ten times in the insert to show the relative intensities of the two absorption peaks.

Quantitative evaluation of the data shown in table 1 indicates that the absorption is quite constant per peptide bond. Thus the polyglutamic acid containing 600 peptide bonds has an absorptivity 600 times greater than alanylalanine and alanylglycine. Similarly, the polylysine preparation, having about 85 peptide bonds, has an absorptivity about 110 times that of alanylalanine.

To further study the relationship of absorbancy to the number of peptide bonds, bovine serum albumin and gelatin were hydrolyzed, and the absorbancies were measured as a function of time of hydrolysis. The acid hydrolysis of gelatin went to completion as shown in figure 9. The enzymatic hydrolysis of gelatin by pepsin proceeded as far as the enzyme would carry it as shown in figure 10. Similar results were obtained with bovine serium albumin as shown in figure 11.

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Figure 8. Absorption Spectra at pH 1-Bovine Serum Albumin

Figure 9. Acid Hydrolysis of Gelatin (Absorbancy vs. Time)

Figure 10. Pepsin Hydrolysis of Gelatin (Absorbancy vs. Time)
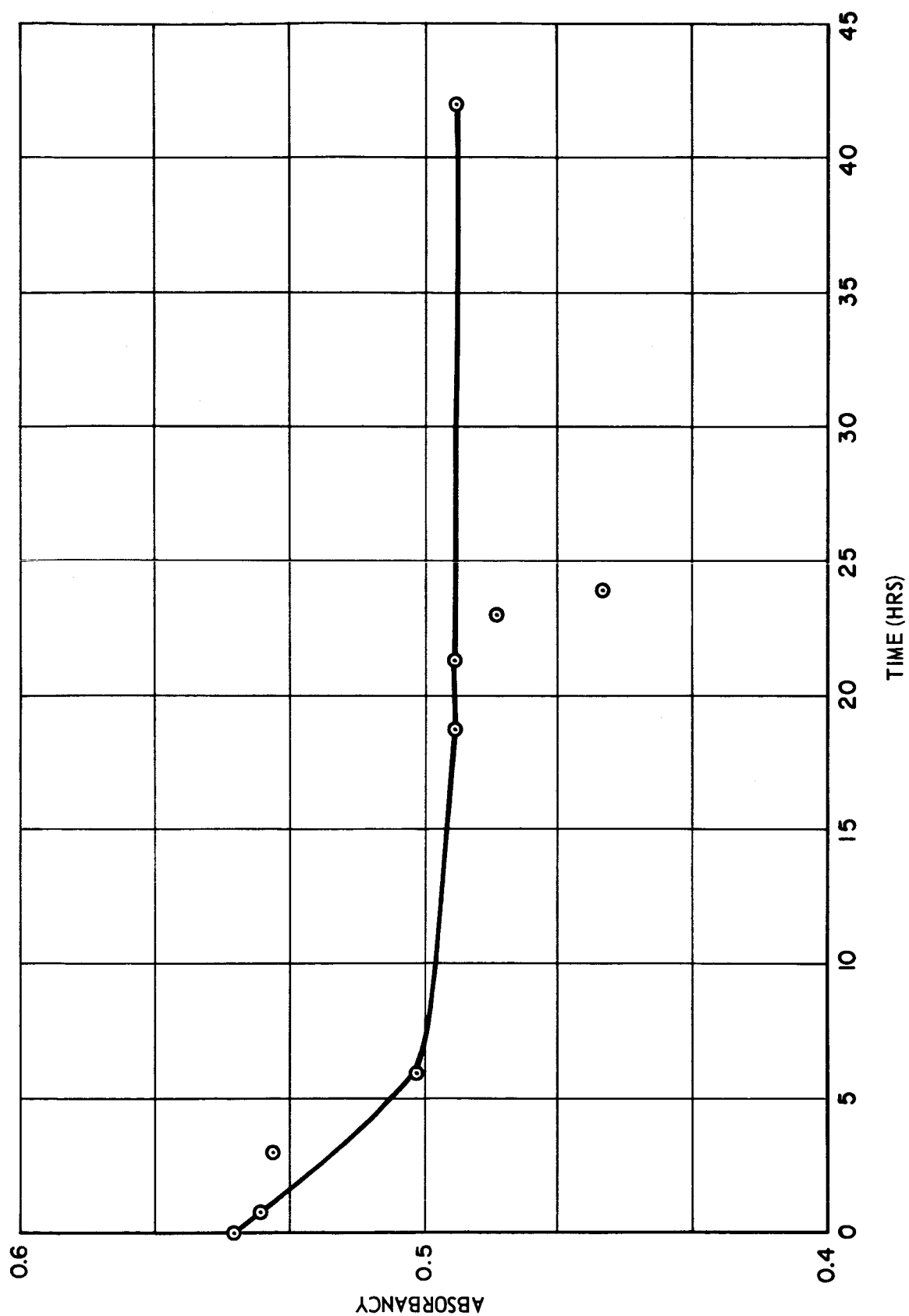
Figure 11. Pepsin Hydrolysis of Bovine Serum Albumin (Absorbancy vs. Time)

TABLE 1

MOLAR ABSORPTIVITY OF PEPTIDES

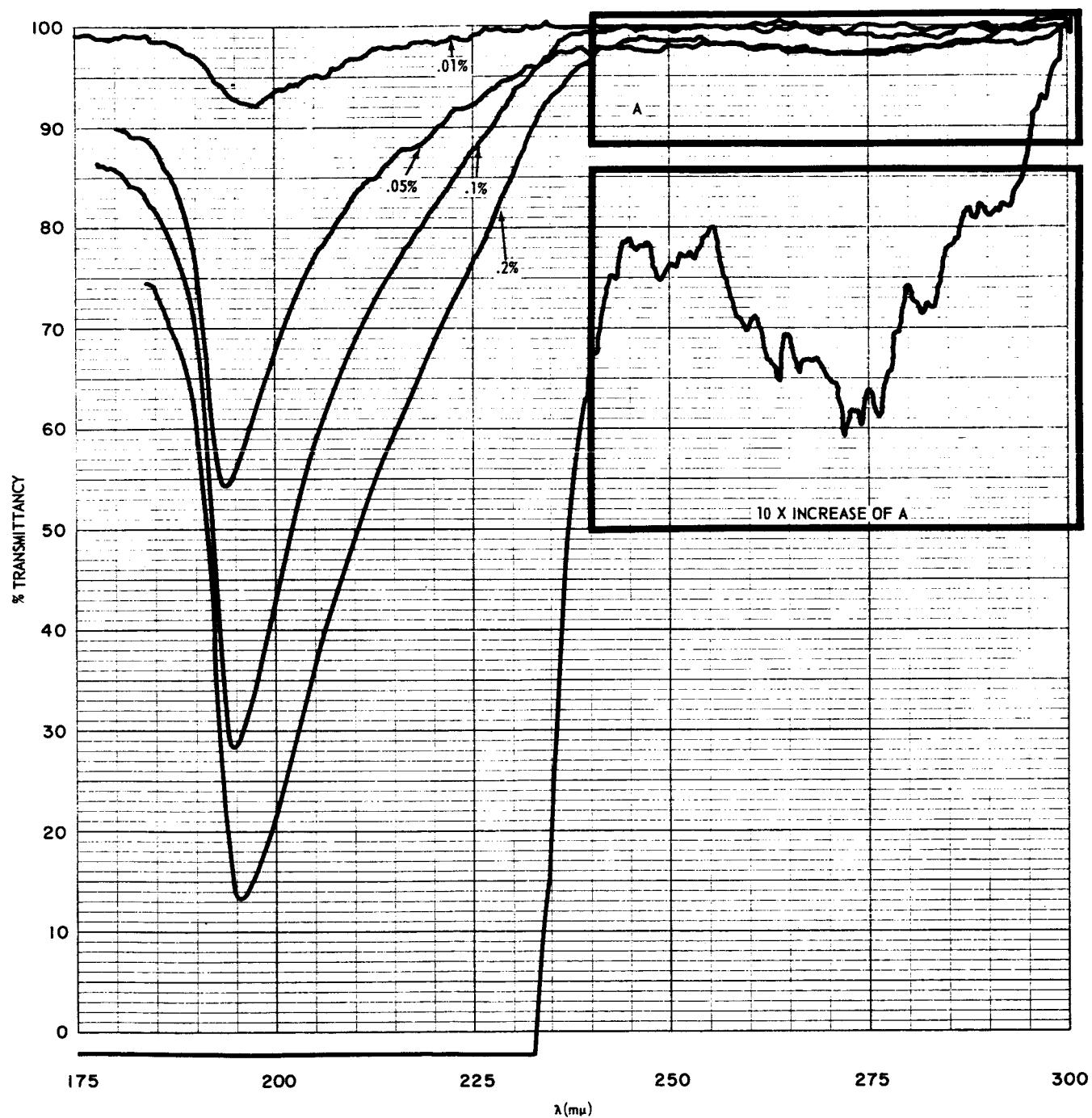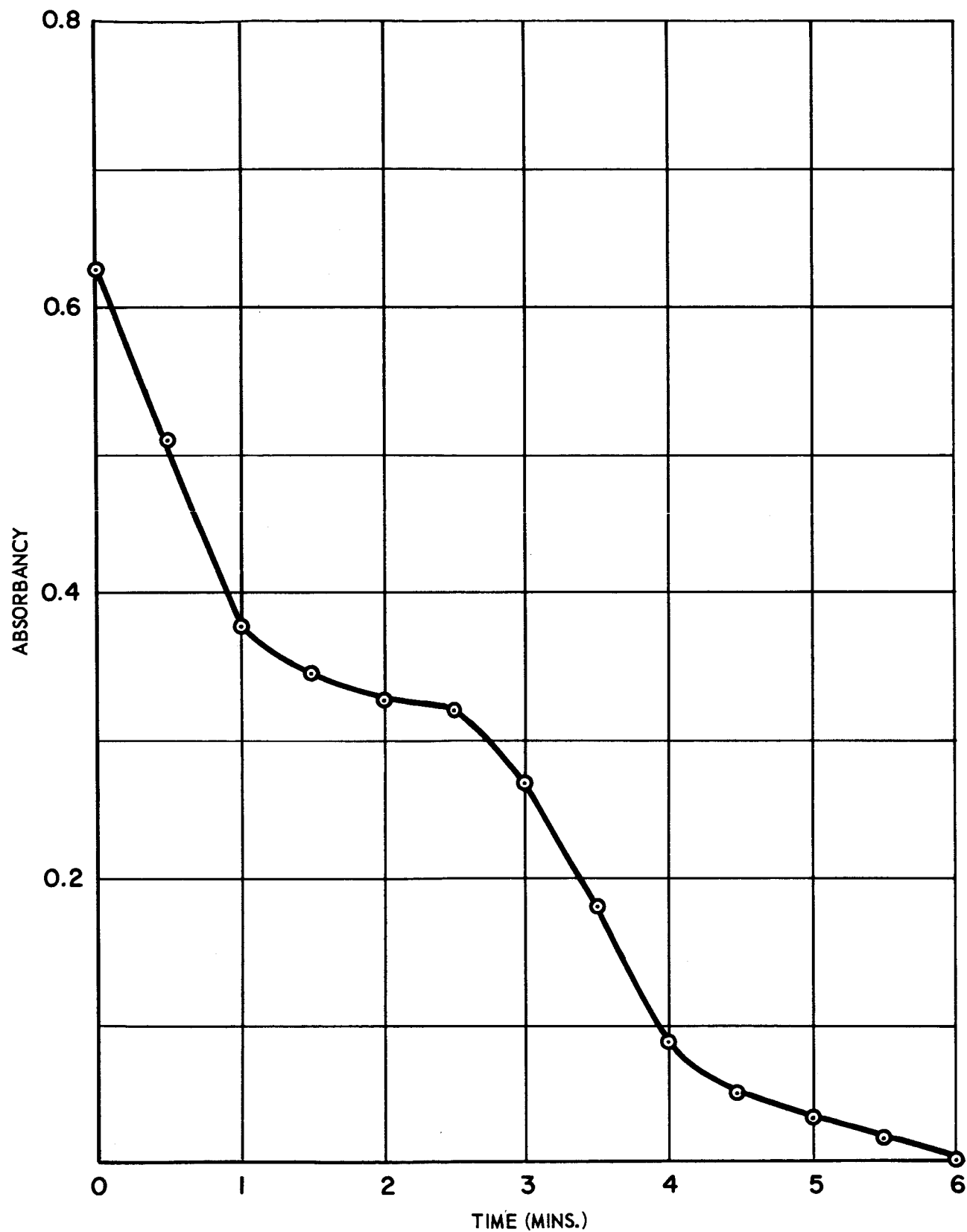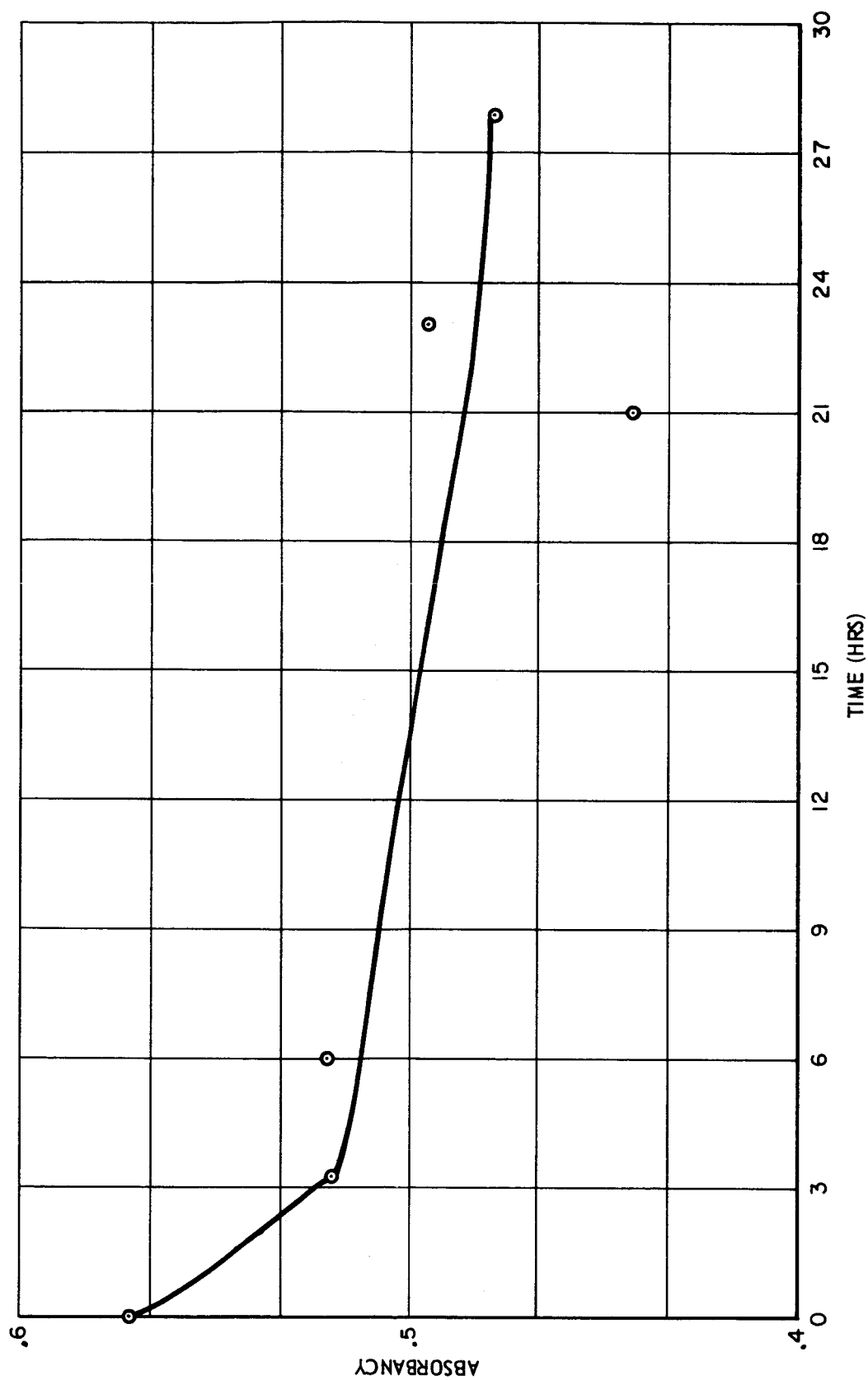| Material	ABSORPTIVITY (MOLAR)					
	pH 1	pH 3	pH 5	pH 7	pH 9	pH 11
DL-alanyl-DL-alanine	1.36×10^3 $*9.1 \times 10^3$	13.4×10^3	15.8×10^3 $*16.4 \times 10^3$	15.7×10^3	17.7×10^3 $*13.9 \times 10^3$	$.71 \times 10^3$ $*6.1 \times 10^3$
DL-alanyl-glycine	1.26×10^3 $*10.2 \times 10^3$	15.6×10^3	16.4×10^3 $*17.2 \times 10^3$	16.2×10^3	12.5×10^3 $*13.4 \times 10^3$	1.01×10^3 $*7.9 \times 10^3$
DL-alanyl-DL-leucine	2.48×10^3 $*17 \times 10^3$	30.4×10^3	26×10^3	24.4×10^3	20×10^3 $*24.4 \times 10^3$	1.21×10^3 $*14.4 \times 10^3$
DL-leucyl-glycyl-glycine	2.43×10^3 $*17.7 \times 10^3$	27.5×10^3	27.5×10^3	26×10^3	20.3×10^3 $*26 \times 10^3$	1.31×10^3 $*11.1 \times 10^3$
DL-alanyl-glycyl-glycine	1.48×10^3 $*15.4 \times 10^3$	26.8×10^3	24.4×10^3	23×10^3	16.5×10^3 $*20 \times 10^3$	1.22×10^3 $*9.35 \times 10^3$
Poly-L-lysine	1.66×10^5 $*15.5 \times 10^5$	18.4×10^5	22.4×10^5 $*24.2 \times 10^5$	16.5×10^5	12.5×10^5 $*19 \times 10^5$	$.77 \times 10^5$ 10.4×10^5
Poly-glutamic acid				10×10^6		
Poly glycine	$**2.9 \times 10^5$					
Bovine albumin	1.0×10^6 $*6.25 \times 10^6$	8.3×10^6	1.8×10^6	7.8×10^6	6.0×10^6 $*6.9 \times 10^6$	$.66 \times 10^6$ $*4.9 \times 10^6$

 *   Indicates peaks other than at 190 wavelength

**   pH was less than pH 1

3.2 Interferences

Spectrophotometric studies were carried out on a wide variety of substances for the purpose of exploring solvents other than water and ascertaining the nature of substances that absorb in the same region as the peptide bond. Representative compounds and the wavelength range of absorptions are shown in table 2. The wavelength regions shown in the table are bounded by the wavelengths at which the absorbancies are 0.3.

3.3 Extracts of Local Soil and Sand

It was found that aqueous extracts of silica, clean glass, and washed ignited sand all have a relatively narrow absorption band at 177-178 mu as shown in figure 12.

The spectrum of a water extract of unwashed sand (source-Potomac river) showed an absorption peak at 180 mu as shown in figure 13. When the pH of this extract is adjusted to a value of 1, the absorption peak shifts to 193 mu, and the absorbancy decreases.

When the extract is hydrolyzed in hot hydrochloric acid, the absorption peak shifts to 197 mu and the absorbancy decreases. Upon adjusting the hydrolyzed extract to pH 1, the absorption peak shifts to 202 mu, and the absorbancy decreases. A comparison of the water extract and hydrolyzed extract at pH 1 is shown in table 3. It will be noted that in all cases, hydrolysis of the sand extract results in a shift of the absorption maximum to longer wavelengths and in a decrease in absorbancy.

Similar experiments carried out with local soil are shown in table 4, and a typical spectrum is shown in figure 14. It will be noted that the absorption of soil extracts is affected by hydrolysis in a manner similar to that of sand. The narrowing of the absorption band seen in figure 14
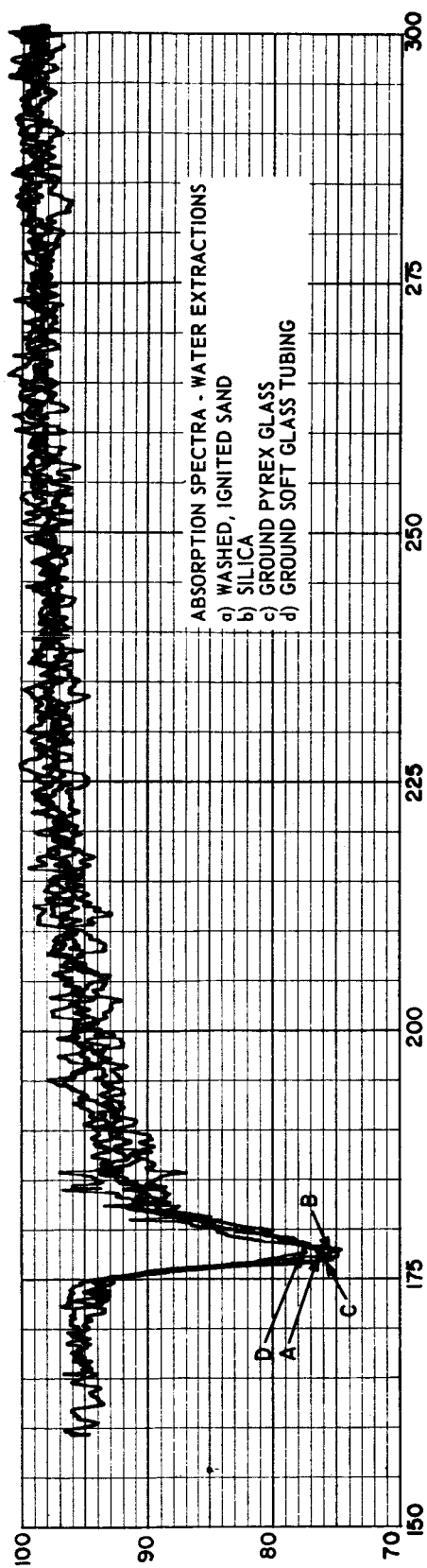
Figure 12. Absorption Spectra – Water Extractions

ABSORPTION SPECTRUM - POTOMAC RIVER SAND

a) WATER EXTRACTION - POTOMAC
b) SAMPLE (a) ADJUSTED TO pH 1
c) SAMPLE (a) HYDROLYZED IN HCl
d) SAMPLE (c) ADJUSTED TO pH 1
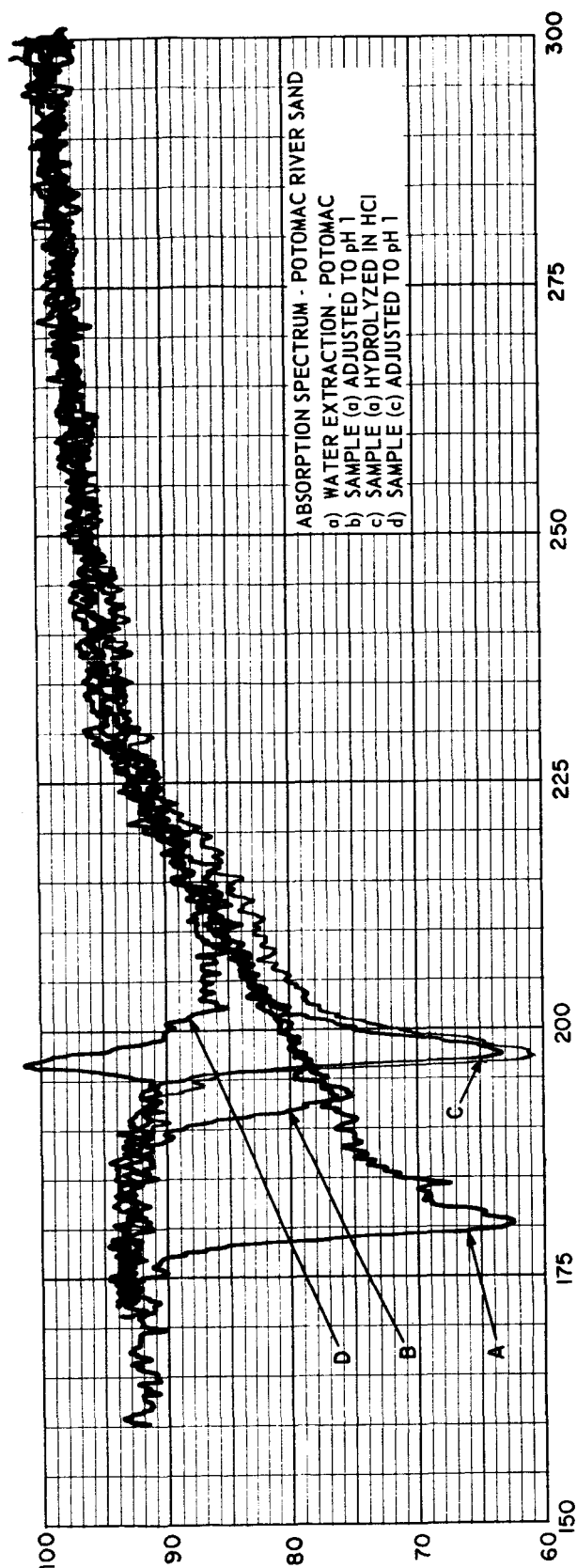
Figure 13. Absorption Spectrum - Potomac River Sand

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ABSORPTION SPECTRUM -
WATER EXTRACTION OF SOIL

a) WATER EXTRACTION
b) SAMPLE (a) ADJUSTED TO pH 1
c) SAMPLE (a) HYDROLYZED WITH HCl
d) SAMPLE (c) ADJUSTED TO pH 1
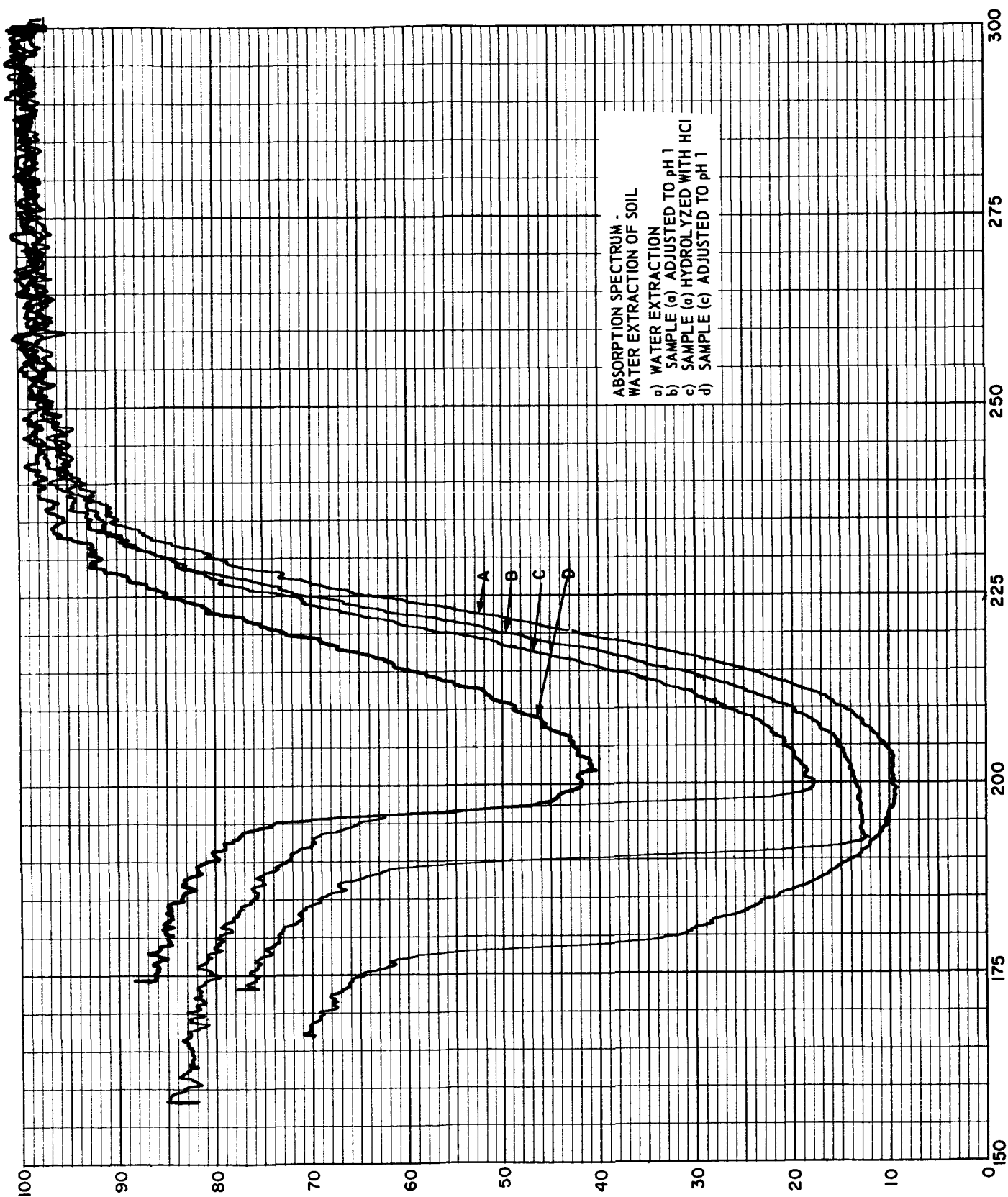
Figure 14. Absorption Spectrum - Water Extraction of Soil

TABLE 2

WAVELENGTH RANGE OF ABSORPTIONS

Substance	Region of Absorption (mu)
Benzene	175 - 270
Ethanol	175 - 190
Diethyl ether	178 - 200
Isopropyl ether	175 - 260
Butyl alcohol	173 - 245
Cyclohexane	178 - 220
Formaldehyde	178 - 225
Formamide	175 - 300
Hydrogen Peroxide (6%)	175 - 250
Ethyl acetate	175 - 262
Dichloromethane	177 - 200
Acetone	177 - 200
Acetonitrile	178 - 197
Butyl acetate	175 - 225
N.N. Dimethyl formanide	175 - 275
Glycerol	177 - 225
Ethylene glycol	177 - 250
Isopropanol	180 - 225
Acetic Acid	175 - 255
Petroleum ether	176 - 260
1, 4 Dioxane	175 - 262
Methanol	178 - 235
Carbon tetrachloride	178 - 245

TABLE 3

Effect of Acid Hydrolysis on the Absorption Maximum of Aqueous Extracts
of Sand

Sample		Absorption Maximum (mu)	Absorbancy at Maximum
#1	sand	193	.119
	Hydrol.	202	.065
#2	sand	193	.180
	Hydrol.	197	.086
#3	sand	194	.065
	Hydrol.	205	.018
#4	sand	194	.070
	Hydrol.	205	.020

TABLE 4

Effect of Acid Hydrolysis on the Absorption Maximum of Aqueous Solutions of Soil

Sample	Absorption Maximum (mu)	Absorbancy at Maximum
#1	194	1.045
Hydrol.	196	.508
#2	193	.876
Hydrol.	203	.376
#3	200	.853
Hydrol.	210	.070
#4	200	.768
Hydrol.	203	.228
#5	203	.960
Hydrol.	220	.102

occurred in all replicate experiments. The water extract regularly shows a very broad band. Upon adjusting the pH with hydrochloric acid, the absorption band becomes narrower on the short wavelength side. Further narrowing is observed upon hydrolysis.

Extracts of sand and soil were made with several organic solvents. Although these solvents absorbed in the far ultraviolet, it was possible to obtain spectra with the double-beam spectrophotometer by using the solvent itself in the reference cell. Figure 15 shows the spectrum obtained with a petroleum ether extract of soil. It is immediately apparent that the absorption band is sharper than that obtained with water. There is also some indication of better resolution at 197, 200, and 203 mu than was obtained with water. A generally similar type of spectrum is obtained in acetonitrile as shown in figure 16. On the other hand, extraction with methanol gives a broader band with less resolution resembling that of the aqueous extraction as shown in figure 17.

The effect of various methods of extracting the sample was studied in order to ascertain whether an extraction procedure that can practically be integrated into an automated remotely operated instrument would yield a satisfactory sample. The methods used were: (1) stirring with a magnetic stirrer, (2) stirring with a gas stream, and (3) extracting by pumping a liquid through the sample in a closed system. The results of these experiments indicated that all the extraction methods, that were tried, yielded samples that were essentially identical with respect to their spectrophotometric properties.
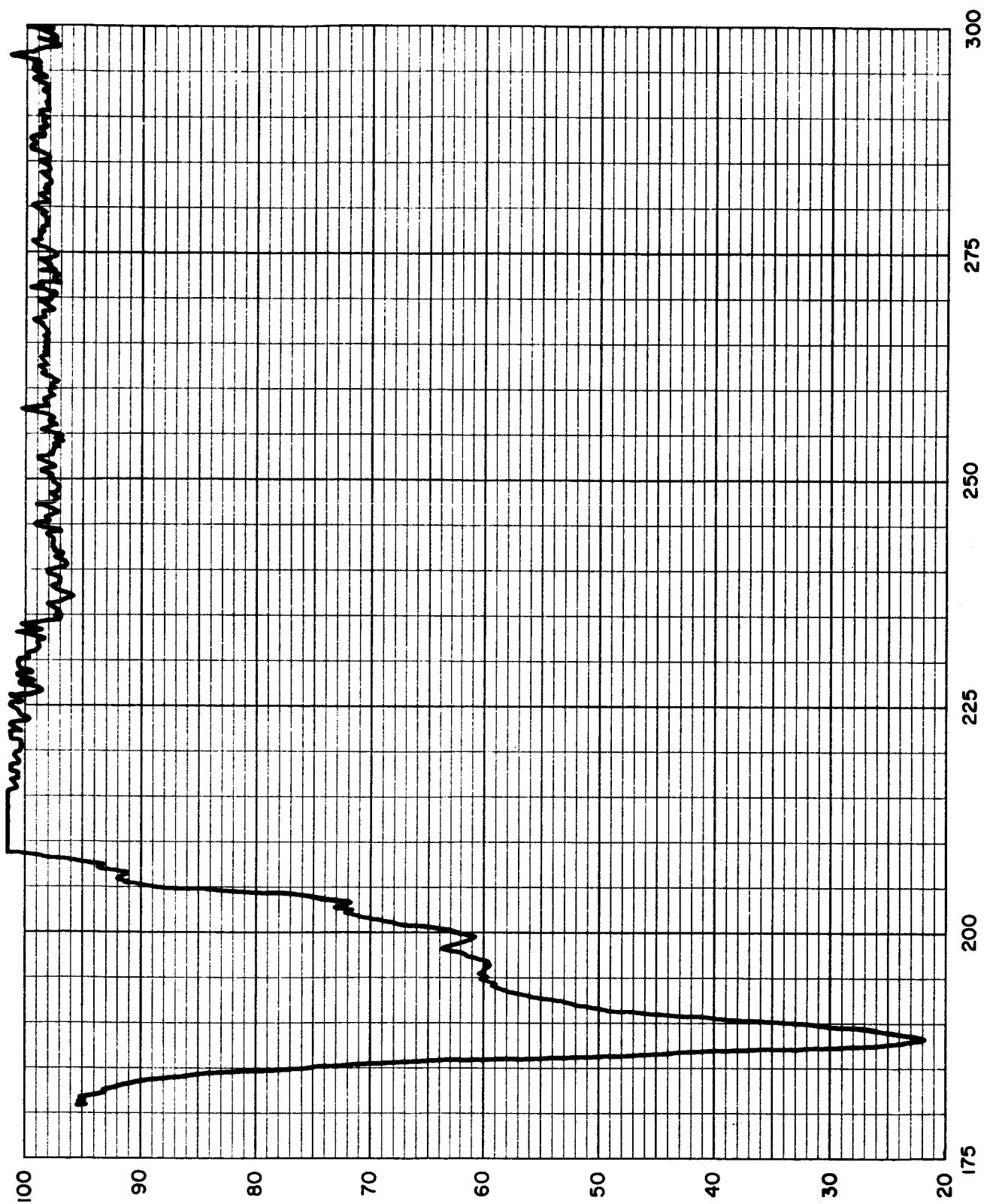
Figure 15. Absroption Spectrum Petroleum Ether Extraction of Soil

R7748
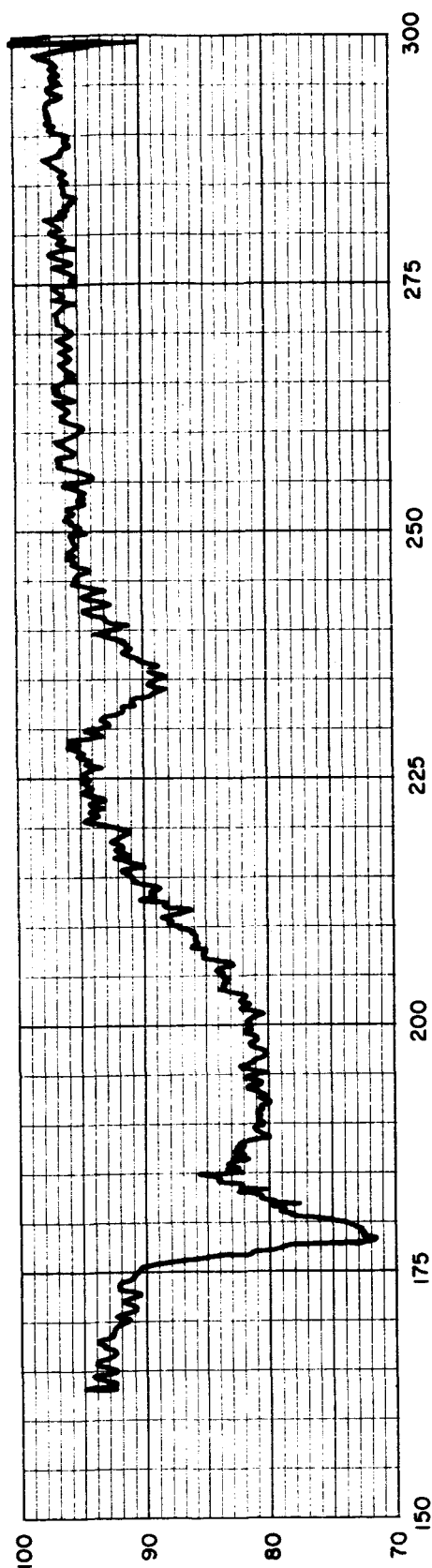
Figure 16. Absroption Spectrum Acetonitrile Extraction of Soil

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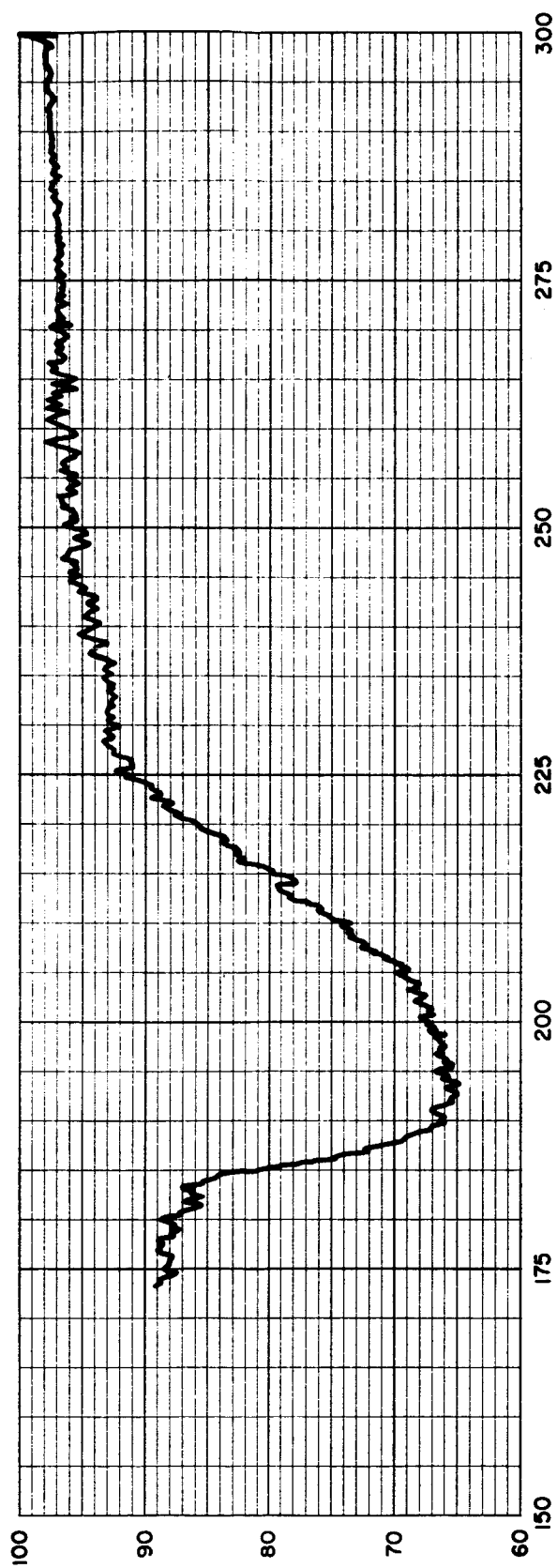
Figure 17. Absroption Spectrum Methanol Extraction of Local Soil

3.4 Whole Organisms

Experiments were carried out with staphylococcus aureus suspended in distilled water with and without hydrochloric acid. As shown in figure 18, the absorption maximum in distilled water was at 1780A°. However, a shift to 1920A° was observed upon the addition of hydrochloric acid.
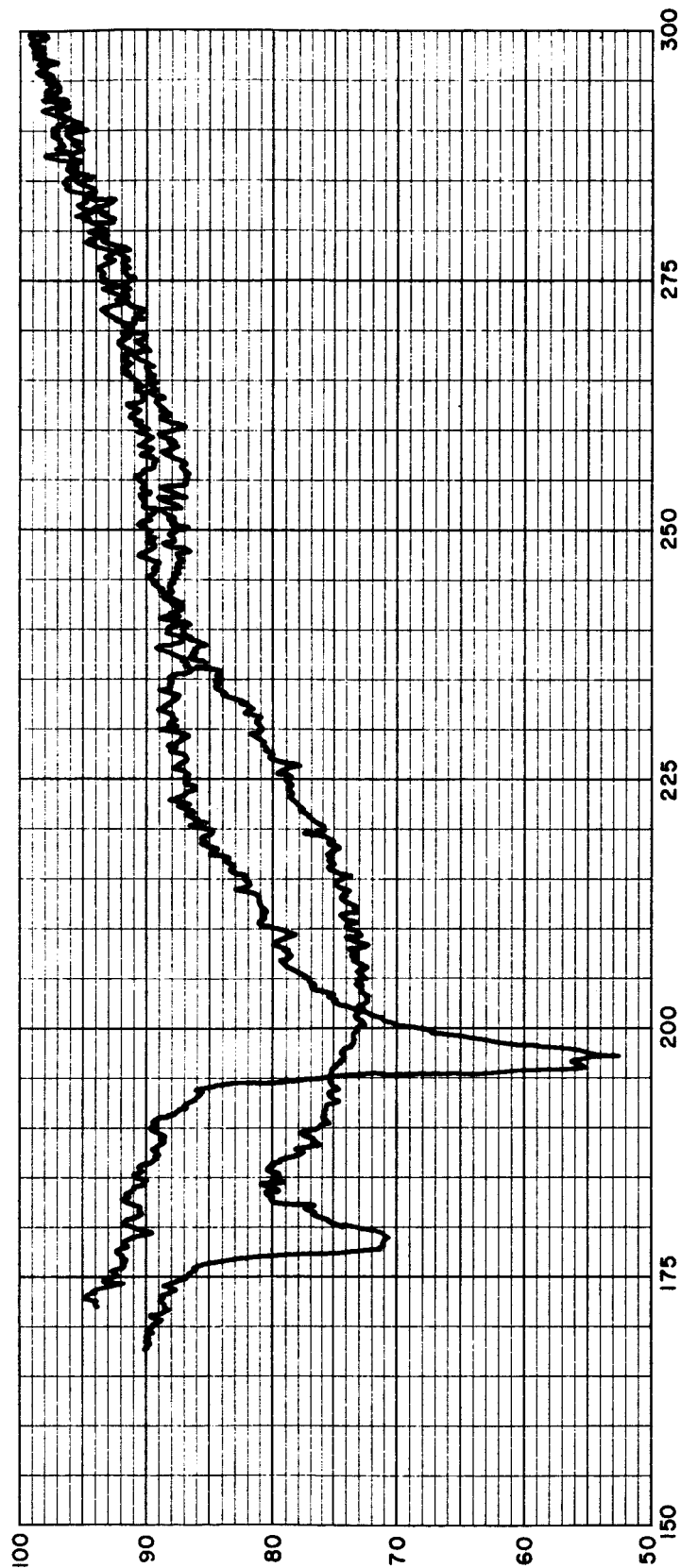
Figure 18. Absroption Spectra - Suspensions of Staphylococcus Aureus

4. DISCUSSION

The availability of synthetic quartz that is transparent to the vacuum ultraviolet has made the use of spectrophotometry in this region of the spectrum entirely practical. The problem of eliminating absorption by oxygen is met quite simply in the laboratory by use of the vacuum or by flushing with nitrogen. However, it is expected that neither of these steps would be necessary in an instrument on Mars since the concentration of oxygen in the atmosphere of that planet is very low.

The results of the program constitute strong evidence for the hypothesis that the absorption of far ultraviolet radiation is due to the presence of the peptide bond. Absorption in the 185-190 mμ region was observed in all the dipeptides, polypeptides, and proteins that were studied. The Beer's low plots indicate that the results are due to true absorption and not to a process such as light scattering which may be confused with absorption. The results of the protein hydrolysis experiments lend further weight to the basic hypothesis.

The experimental results make it unnecessary to carry out specific experiments on the relationship of such factors as the extent of denaturation to the spectrophotometric property under investigation. Acid hydrolysis at a relatively high temperature undoubtedly results in denaturation before degradation takes place. Yet, the denaturation did not seem to affect the spectrophotometric measurement.

The lower limit of detection depends on the molar absorptivity, the solvent system, and the instrumental sensitivity. Thus, substituting the molar absorptivity for a single bond (10^4 from table 1) in the Beer-Lambert

equation and assuming a path length of 0.1cm, a concentration of 10^{-5} moles/liter would be detectable in an instrument that will give a reliable absorbancy measurement of 0.04. The absolute quantity detectable in 1.0ml would then be 15^{-8} moles.

Reference to table 1 indicates that a greater molar absorptivity is obtainable at higher pH values. However, it was also pointed out that aliphatic amino acids and the carboxyl ion, in general, also absorb at the wavelength of interest. In order to avoid this "false" positive, it is more profitable to sacrifice the small increase in sensitivity available at higher pH values.

The long list of interferences, (i.e. nonpeptides absorbing at the wavelength of interest) points to a need for taking precautions against the probability of a false positive signal. It is not known if any of these substances or those reported in the literature[7] are present on Mars. Nevertheless, a reliable approach to the problem of extraterrestrial life detection must be based on the elimination of as many possible sources of error as can be anticipated. Such an approach may be based on the hydrolysis of the sample. All the experiments carried out with acid hydrolysis on proteins, local soil, and Potomac river sand showed a detectible difference between the absorption by the hydrolyzed and unhydrolyzed samples.

The experiments with bacterial suspensions are particularly interesting because the absorption measurements were made on whole organisms. In such a case, it might be expected that the attenuation of the incident beam might reasonably be due to light scattering rather than to true absorption. However, the spectrum indicates that this is not the case. If the signal were due to light scattering, the absorption would tend to decrease as the wavelength

is lowered, since the intensity of scattered light varies inversely with an exponential function of the wavelength. Since the absorption is in the form of a discrete band, it may then be assumed that the attenuation of the incident beam is due to absorption.

The spectral data obtained on a wide variety of biological materials leave little doubt that the peptide bond can be detected by far ultraviolet absorption techniques. Since the presence of peptides is a very strong indication of the presence of biological systems, the detection of peptides may be regarded synonymously with the detection of life. The feasibility of detecting extra-terrestrial life by means of the techniques described must be considered in terms of a biological system in the presence of other substances that absorb in the same ultraviolet region as the peptides. By integrating the information obtained in this program, one may derive constraints on which a practical instrument may be based. Thus, the consideration of pH effects, interfering substances, and the results of hydrolysis, indicate the conditions necessary for the feasibility of applying far ultraviolet spectrophotometry to detection of extraterrestrial life.

5. CONCLUSION

The results of the research program indicate that the detection of extraterrestrial life is feasible by application of far ultraviolet spectrophotometry. The instrument should be a double-beam instrument with a broad-band monochromater spanning the region between 185 mμ and 220 mμ. The sampling system should be capable of collecting a sample of soil or atmospheric aerosol and introducing it into a processor. The processor must be capable of extracting the sample with water, and then adding a volume of hydrochloric acid. The extract must then be divided into two aliquots, one of which is subjected to hot-acid hydrolysis. The unhydrolyzed sample is to be introduced into the reference cell, and the hydrolyzed sample is introduced into the sample cell for spectrophotometric measurement.

Such an instrument is entirely feasible, and Melpar has submitted a proposal for the development of a laboratory model.

6. RECOMMENDATIONS FOR FUTURE WORK

In view of the positive results that were obtained, it seems prudent to develop, design, and construct a laboratory model of a life-detection instrument. Melpar has proposed to undertake such a program and the general aspects of the instrument are described in the following pages.

6.1 Design Considerations

The design of a laboratory model instrument for investigating the feasibility of automatically collecting and processing a sample, and then, carrying out a spectrophotometric analysis is governed by several factors. Some of these factors are basic to the general concept; others are concerned with engineering constraints.

It is assumed that the prototype that succeeds this model will be a complete entity and will not be dependent on other instruments for major component functions. Thus, the model instrument will collect its sample, carry out all necessary processing steps, and then perform the spectrophotometric analysis for peptides.

The breadboard design must be designed with a forward thought toward the trend; advanced models will follow. Therefore, it is important to choose components that will be reasonable precursors of components to come later. It is also necessary that the overall design be such as to lead into later designs in terms of power consumption, vibration resistance, weather resistance, etc.

Detailed consideration of the relationship between this device and other possible components of the eventual instrument package is premature in the breadboard stage. However, consideration will be given to the

problems involved in the ultimate integration of this device, with the optical rotation device being considered. Since both instruments have similar sample handling problems and somewhat similar optical components, it is entirely reasonable to design the breadboard with thought given to the eventual integration of the two instruments.

The laboratory model that is prepared here is expected to conform to the following size, weight, and power consumption estimates.

	Subsystem Unit	Size Cu. In.	Weight Pounds	Power Watts
1.	Optics	72	3.00	.25**
2.	Electronics	14	.30	.70
3.	Sample Collector	30	1.00	--
4.	Sample Processor	14	.50	--
5.	Pneumatic Supply	25	.80	--
6.	Programmer	14	.30	--
7.	Squibs and Exothermic Cartridges*	2	.10	.15
8.	Module Frame, etc.	--	.30	--
	Assembled Instrument	171	6.30	1.10

* Using RC network supply

** May possibly be reduced to 0.1 w

6.2 Sampling

A number of methods might be considered for obtaining the surface sample which is to be analyzed by UV absorption techniques. These would include: drilling a hole, scraping fragments into a container, firing

out strings coated with adhesive, and collecting aerosolized particles out of the atmosphere by a vacuum source.

Since an instrument package may contain a number of instruments for analysis of surface samples, it might be advantageous to use a central sample-collection system which would distribute parts of a large sample to the various instruments. A detailed design of a collection system will require more intimate vehicle-instrument interface knowledge, such as location of the instrument in the complete package and clearance to the Martian surface for possible sampling probes.

Acquisition of a surface sample for the instrument requires some additional consideration such as the location and consistancy. Based on the premise that elementary forms of life are more likely to exist slightly below the surface than on the surface because of ultraviolet radiation, the sample should include subsurface particles. Explosive charges may be dropped to raise a dust cloud and fragments some of which would settle on or near the instrument package, where they could be picked up by a vacuum motivated probe. However, this may be difficult because of the low density of the Martian atmosphere.

The mechanical design of the instrument will be based on a sample-collection system in which particles are transported into the extraction chamber by flow of gas in a tube. This is believed to be a practical way to move the sample after it has been picked up and reduced to small particles. Adequate gas flow to transport particles within the instrument package could be obtained from gas bottles.

Design of the instrument is not strongly dependent on the means of delivering the sample. It is felt that any other scheme for delivering the sample to the extraction chamber could be readily accommodated.

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6.3 Processing

A start pulse will be required to begin the instrument operation. If the instrument has been subjected to low temperatures, chemical exothermic reactors will provide enough heat to raise the temperature above $0^{\circ}C$ and, melt the solvent if it is frozen. A thermistor will be strategically located to sense the attainment of a suitable temperature and fire a squib to start the operation. In the event that the starting temperature of the instrument is above freezing, the heaters will be locked out by a second thermistor and operation will commence immediately.

The mechanical-processing system must perform the following functions:

a. Deliver the sample to the extraction chamber.

b. Inject a solvent into the system.

c. Circulate the solvent around the sample particles to extract the chemicals which may exhibit the peptide bond.

d. Acidify the solution.

e. Filter out light-scattering solid particulates.

f. Inject the solution into a sample cuvette and a reference cuvette.

g. Hydrolyze the sample in the reference cuvette.

Figure 19 and figure 20 show two possible schemes for the sample processing system.

It is assumed that the particles to be analyzed will be blown into the extraction chamber by a stream of gas, and caught on a filter. However, this is not an essential factor of the device, and other methods of delivering the sample to the extraction chamber might be considered.

The solvent and acid will be carried in sealed reservoirs which operate on the hydraulic accumulator principle. Pressure on the solvent may be
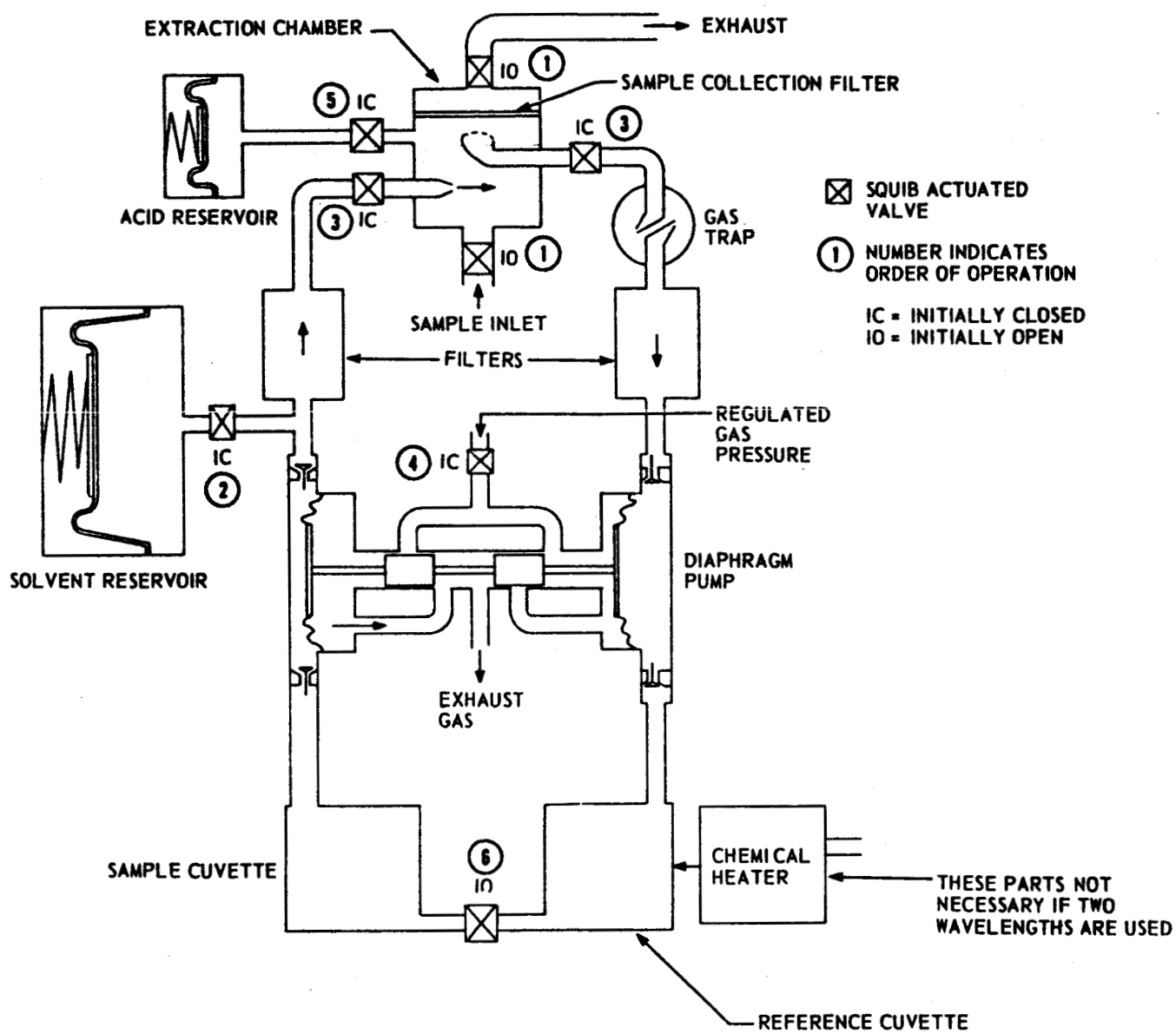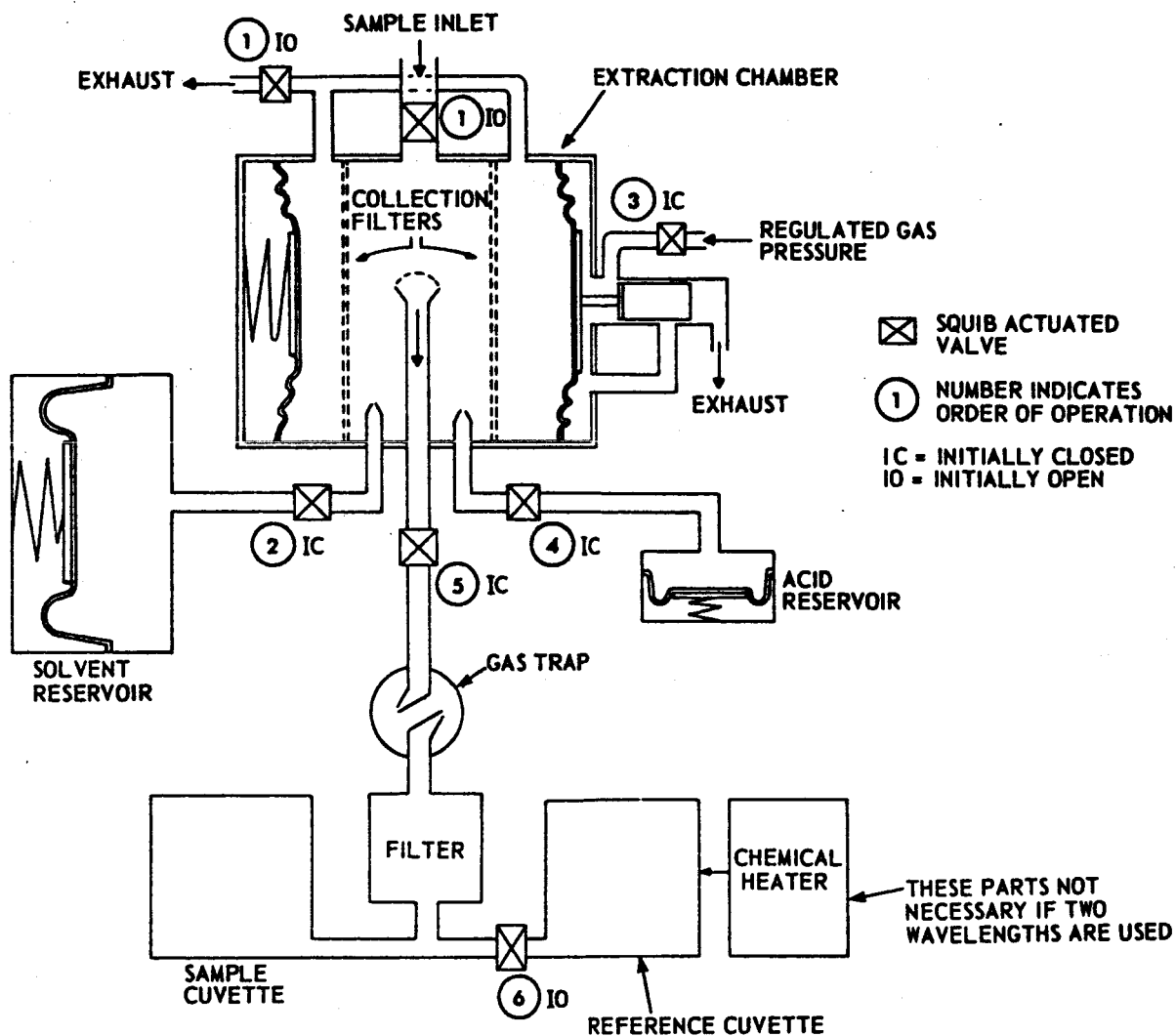
Figure 19. Sample Process System Scheme 1

Figure 20. Sample Processing System Scheme 2

47

provided by either compressed gas or by a spring pressing on a diaphram.

Since the cavity containing the solvent is flexible, freezing will not

cause significant stresses. The solvent will be injected by opening a

squib-actuated valve.

All of the connecting tubing and the cuvettes will be evacuated and

sealed during manufacture so that there will be no resistance to the solvent

entering the system. The solvent reservoir will be designed so that the

pressure inside the system will be about 50 psi after the solvent is injected.

Gas, which is brought into the extraction chamber with the sample, will be

compressed; it will occupy only a small portion of the chamber volume. The

outlet from the extraction chamber to the cuvette will be located at the

center of the chamber so that no gas will be delivered to the cuvette. Any

gas already in the section of pipe inside the extraction chamber will be

caught by a gas trap.

The principal difference in the processing systems of figures 19 and 20

is in the method of circulating the solvent to extract the sample.

In the scheme of figure 19, the solvent is pumped around a loop, and

injected into the extraction chamber to cause turbulent motion of the fluid.

This method of extraction has been tested in Melpar's laboratory and shown

to be practical.

In the scheme of figure 20, the sample is trapped between two filters

in a chamber with flexible diaphragm ends. Gas pressure is admitted period-

ically behind one of the diaphragms to cause the fluid to flow back and forth

through the filters. This scheme appears to be somewhat more simple.

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Selection of the exact scheme to be used will require a detailed analysis of the amount of power required to accomplish the extraction, and will naturally depend on which system can accomplish the operation with the lowest total weight and power consumption. In either case, the valves regulating flow of the gas which causes the pumping action must be designed to have an "over center" action, so that the pump cannot be caught on dead center.

After the extraction has been accomplished, acid will be injected by firing another squib-actuated valve. This may be done by rupturing a glass ampule. Pumping action will continue for a short time to complete mixing the acid into the solvent.

For the scheme of figure 19, the acidified solvent will now be in the cuvettes. For the scheme of figure 20, another squib-actuated valve will be fired, allowing the acidified solvent to be injected into the cuvettes, which were evacuated during assembly of the instrument.

If it is decided to compare hydrolyzed with unhydrolyzed samples, it will be necessary to activate a small, chemical heater to hydrolyze the material in one of the cuvettes.

6.4 Pneumatic Subsystem

Although it is not necessary at this stage, it is planned to provide nonelectric (to conserve electrical power drain) motivation for the various mechanical functions that are required in the sample process system and for actuation of the programmer. A high-pressure spherical gas tank approximately two inches in diameter and a small, pressure regulator may be provided for this purpose. Figure 21 is an illustration of this type of tank that was

WELD SEAM

PRESSURE TANK

SEAL IS INTEGRAL
PART OF TANK

SEALED
FILLING PORT

COMPRESSED
GAS

OUTLET TO REGULATOR

SQUIB ACTUATOR

BEFORE FIRING
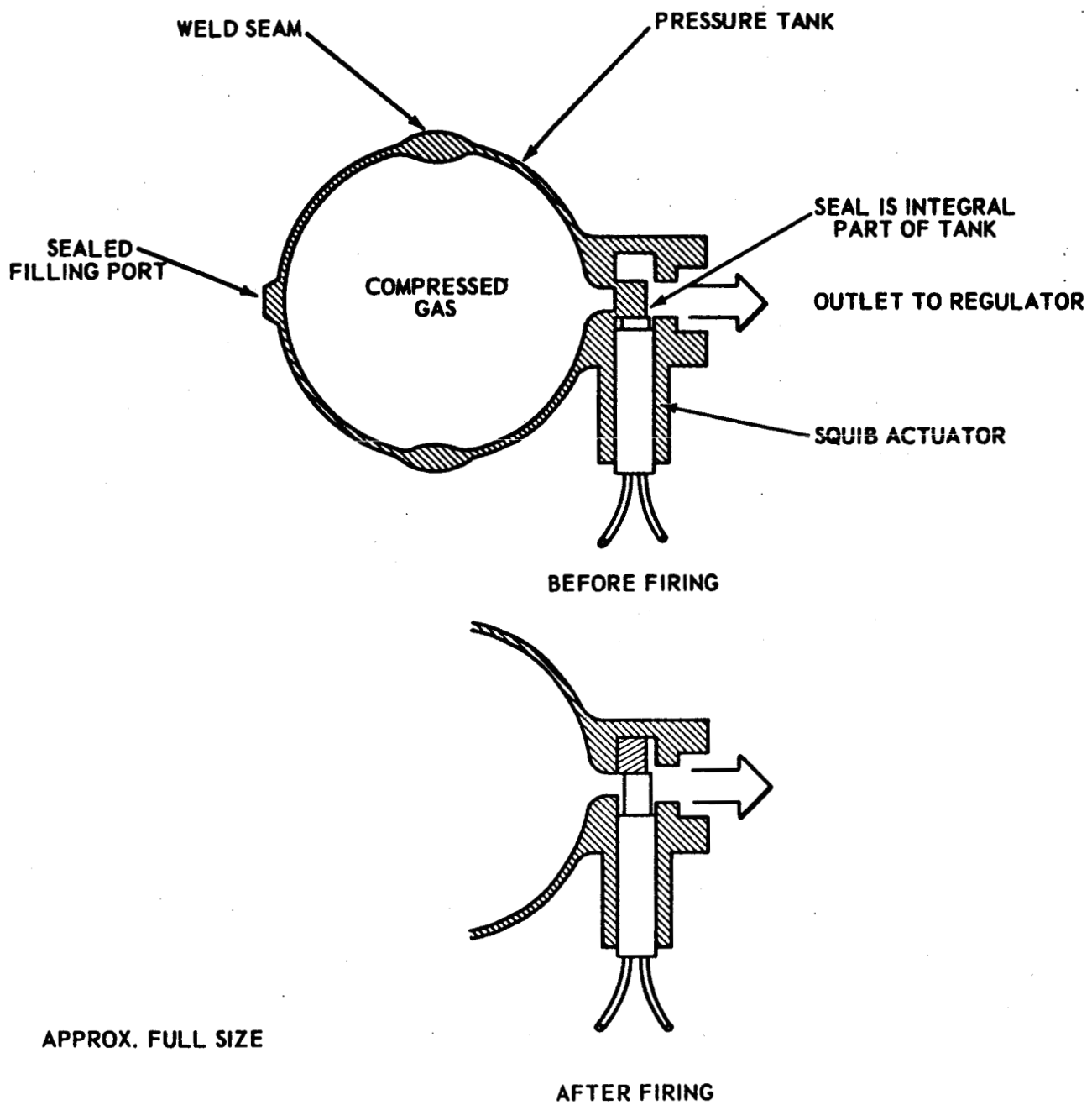
APPROX. FULL SIZE

AFTER FIRING

Figure 21. Pressure Tank and Seal

designed by Melpar for JPL on a previous contract. It was made of titanium and designed for a nominal pressure of 6000 psi with a maximum operating pressure of 18,000 psi under high-temperature conditions. This high-pressure condition would actually be experienced only during sterilization procedure.

Figure 22 illustrates a small, pressure regulator which is available as a commercial item. This would be used in conjunction with the pressure tank to provide a constant pressure output for a sufficient time to perform all necessary functions.

The pneumatically-driven programmer will have a retrofold type of diaphragm driven by air pressure. The exhaust will be controlled by a critical orifice to provide a constant movement of the switch-actuating arm. This unit will be composed of a number of purchased components assembled at Melpar.

6.5 Optical System

The simplest system is a two-detector dual-path instrument. Only one wavelength is required and the output is simply the difference in transmission of the two paths. The general optical schematic of this instrument is shown in figure 23. Light from the source is collimated by the lens, and light between 185-220 mu is passed by the filter to the two sample chambers and detectors. If the beam intensities are initially balanced with no samples, an unbalance, with the samples in place, indicates the presence of peptide bonds in the normal sample.

Many other similar optical schematics might be devised. These include systems using one detector and a vibrating or rotating chopper to allow alternate sampling of the two optical paths. The major problems to be solved in the optical design are the selection or fabrication of an efficent light source and of suitable filters and detectors.
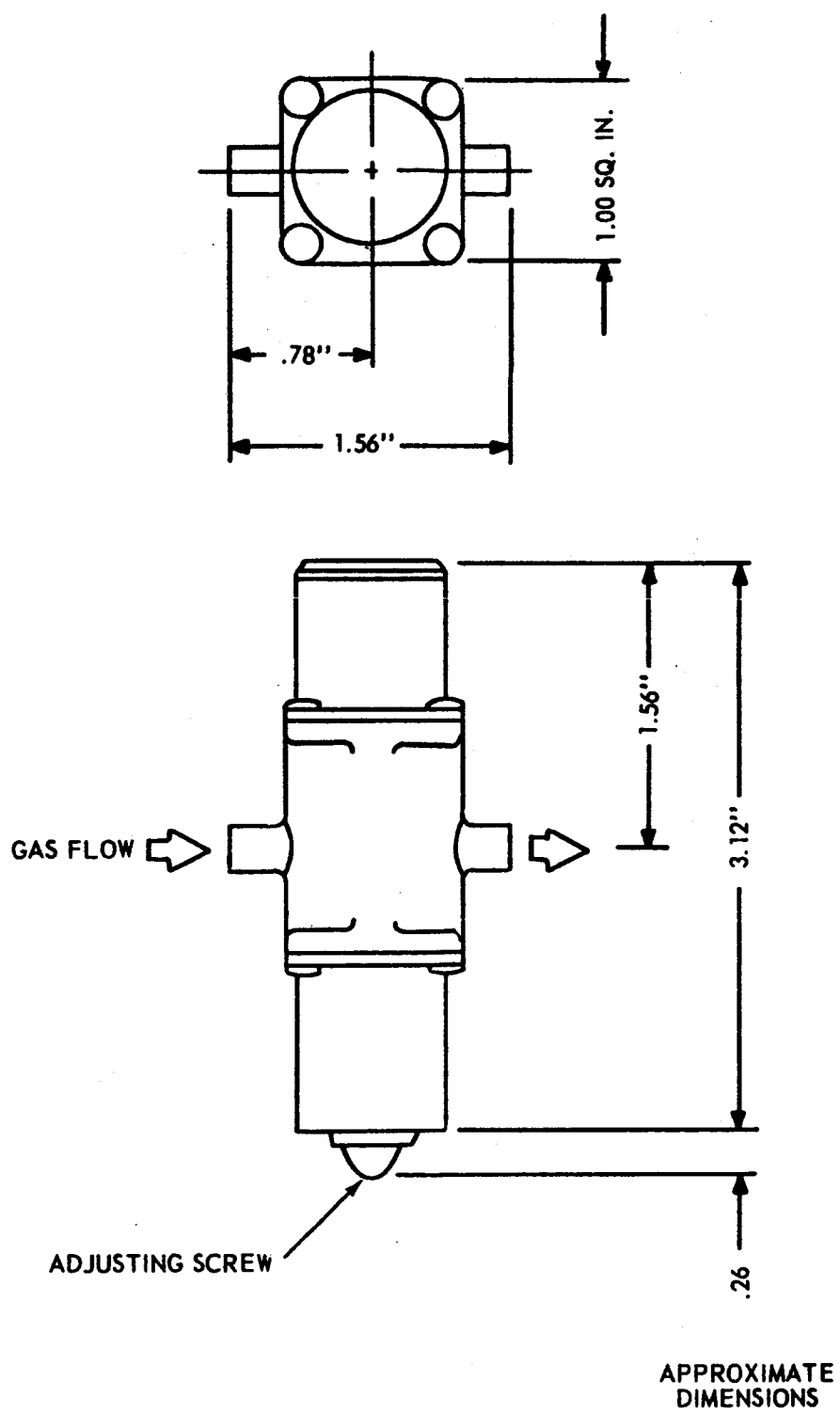
1.00 SQ. IN.

.78"

1.56"

1.56"

3.12"

GAS FLOW ⇨

⇨

ADJUSTING SCREW

.26
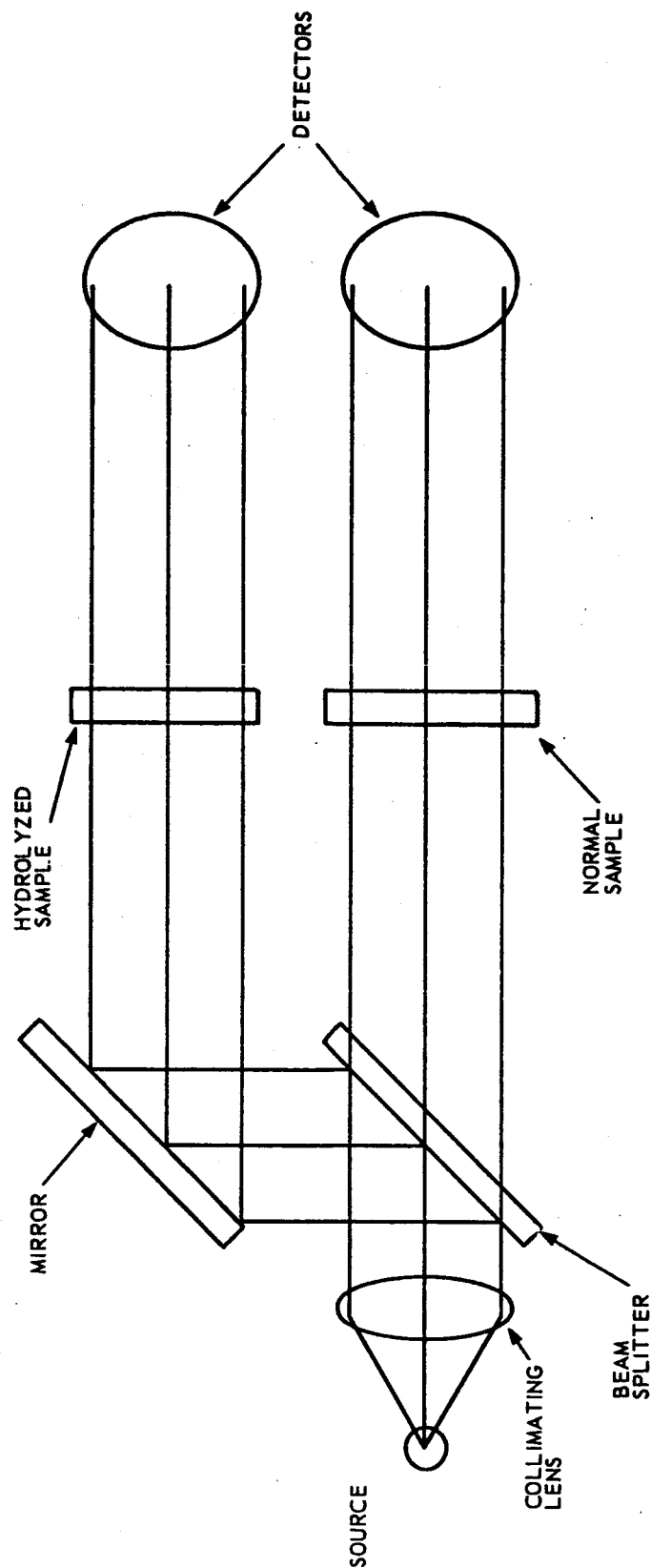
APPROXIMATE
DIMENSIONS

Figure 22. Pressure Regulator

Figure 23. Optical Schematic for Hydrolyzed System

6.5.1 Light Source

Spectrophotometric work in this UV region is usually done with a hydrogen or deuterium lamp giving a continuous spectrum. It is expected that a hydrogen lamp will yield sufficient energy for the purposes of this instrument. In the event that such a lamp proves unable to deliver the required amount of energy, consideration has been given to the use of a pulsed xenon source.

6.5.2 Detector

The detectors will be of the photomultiplier type with the photo-surface material selected to give an adequate response at the desired wavelengths. If the single wavelength 185 mu system is chosen, a tube with a copper iodide photocathode may provide all the discrimination that is needed against the other Hg wavelengths, since this photosurface has very low response above 250 mu. For the two wavelength scheme, a photomultiplier with a rubidium-telluride photocathode would be ideal.[8] Experimental tubes with these and other photocathodes are available and will be investigated to determine exact spectral response, sensitivity, and the influence of the specific detector selection on the filter and on the source energy requirements.

6.5.3 Filters

The spectral transmission characteristics of the filters required for isolating the desired wavelength will depend, as noted above, on the detailed properties of the light source and the detector selected. Generally, it is expected that a broadband interference filter will be completely satisfactory.

6.6 Instrument Construction

The finished model will be extremely rugged, yet light, and capable

of withstanding all of the environmental extremes expected in a space probe.

The sample processing system as well as all other systems will operate

regardless of final orientation (upside down etc.). This is possible

because it is a force fed and completely scaled system. All of the tubing

and diaphragms in contact with the solvent may be made of metal to reduce

the possibility of leaks.

Optical component mounting is a critical design area due to the possi-

bility of breakage under pressures caused by temperature extremes. It is

anticipated that they will be encased in metal frames that nearly match the

coefficients of expansion of the optical components. A low-temperature

silicone rubber mounting, in place of the rigid metal mounting, will be

investigated during the early stages of the development.

Mirrors and lenses will be mounted in silicone rubber and metal frames

in such a manner that expansion due to temperature will not distort the

optical path. The entire optical unit will be assembled as a module.

The electronic system will utilize solid-state components and will be

ruggedly constructed. Techniques for construction of electronic systems for

the Mariner probe are well established and known to Melpar.

Figures 24 and 25 illustrate a squib-actuated valve and an exothermic

cartridge similar in nature to the types of components which will be used in

this system. Both of these items, as well as the programmed exothermic oven,

(figure 26) were developed, fabricated, and tested at Melpar under contract

with JPL. Illustrations of one method of inlet and outlet port sealing are
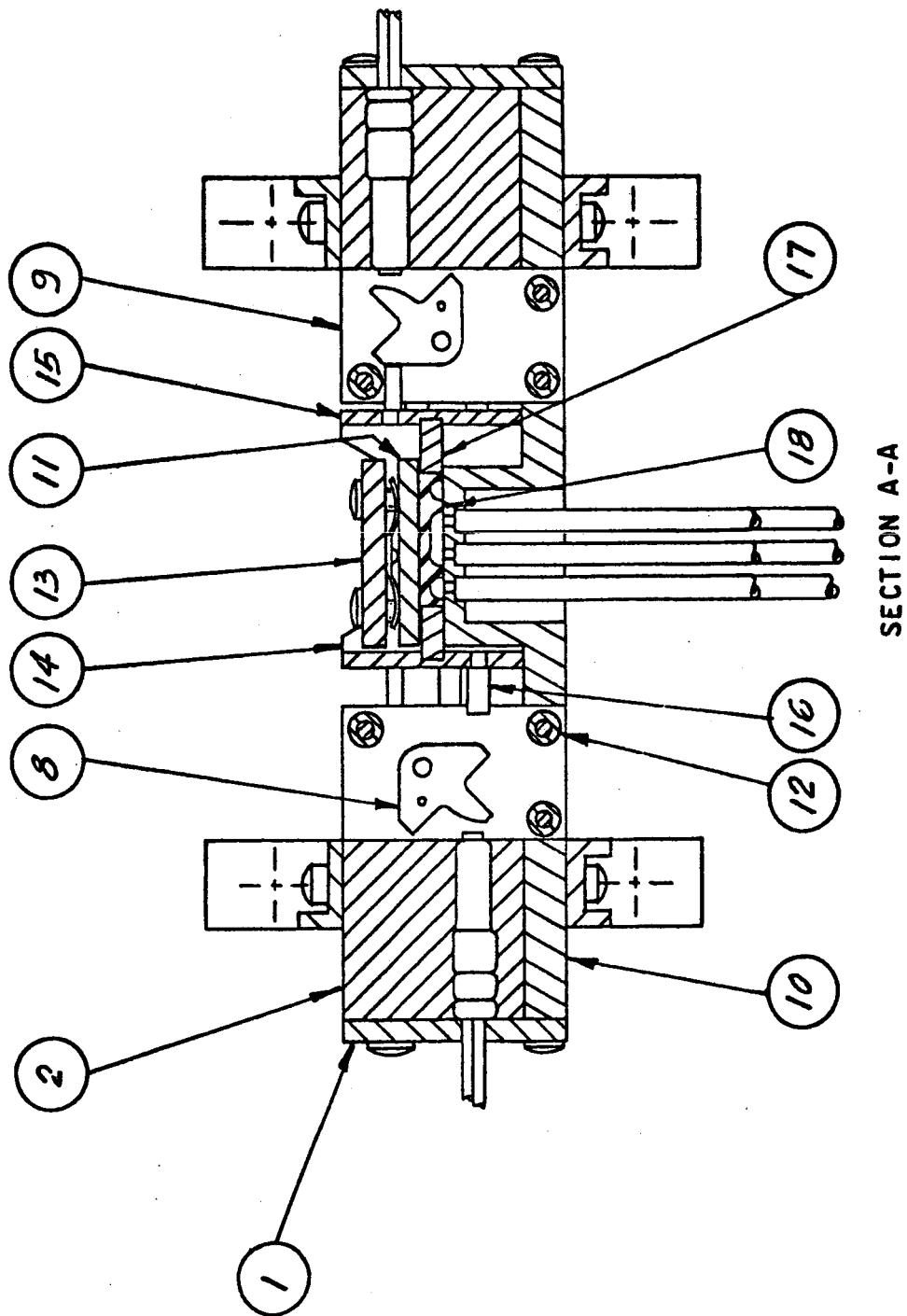
shown in figure 27.

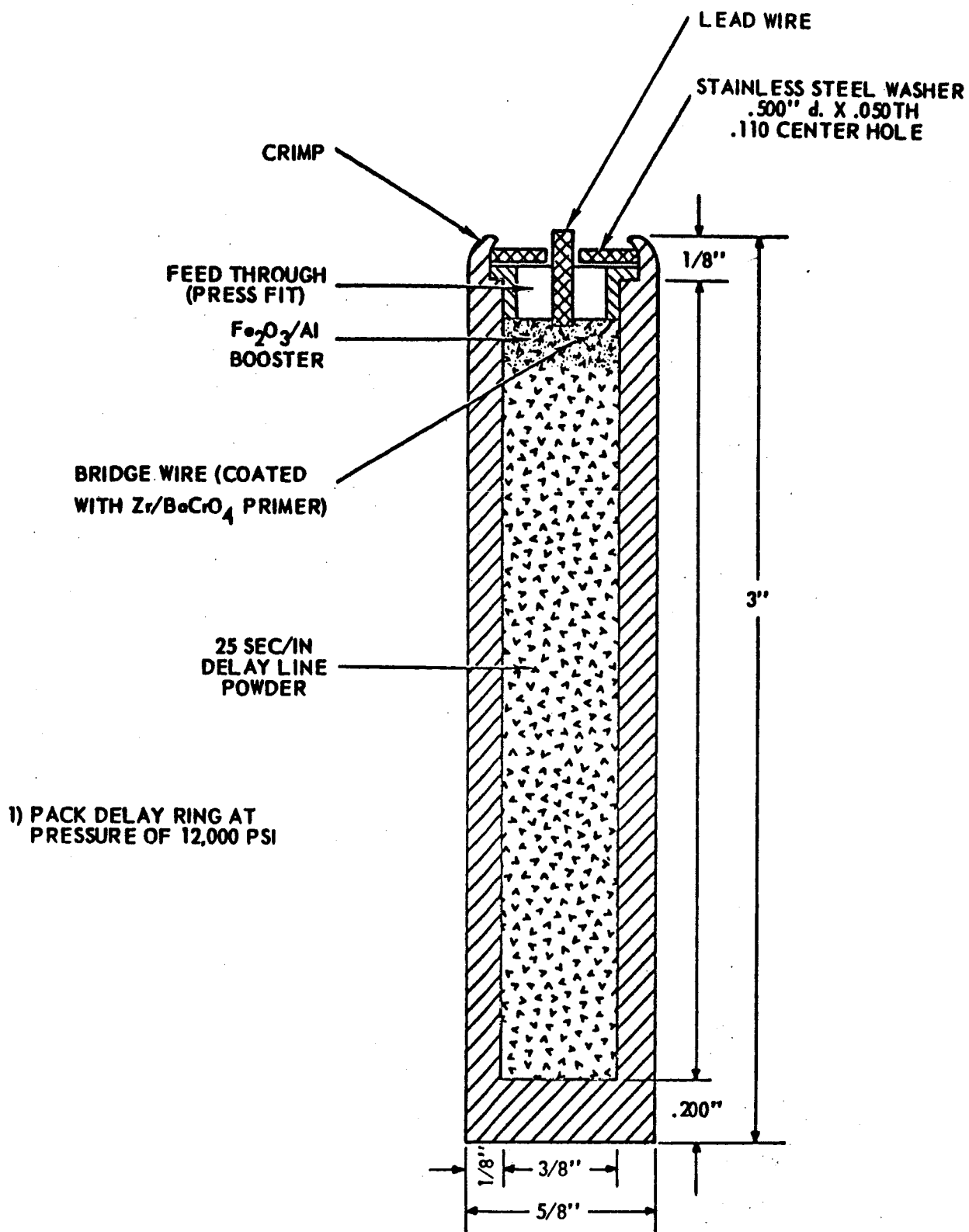Figure 24. Slide Injection Valve (Side View)

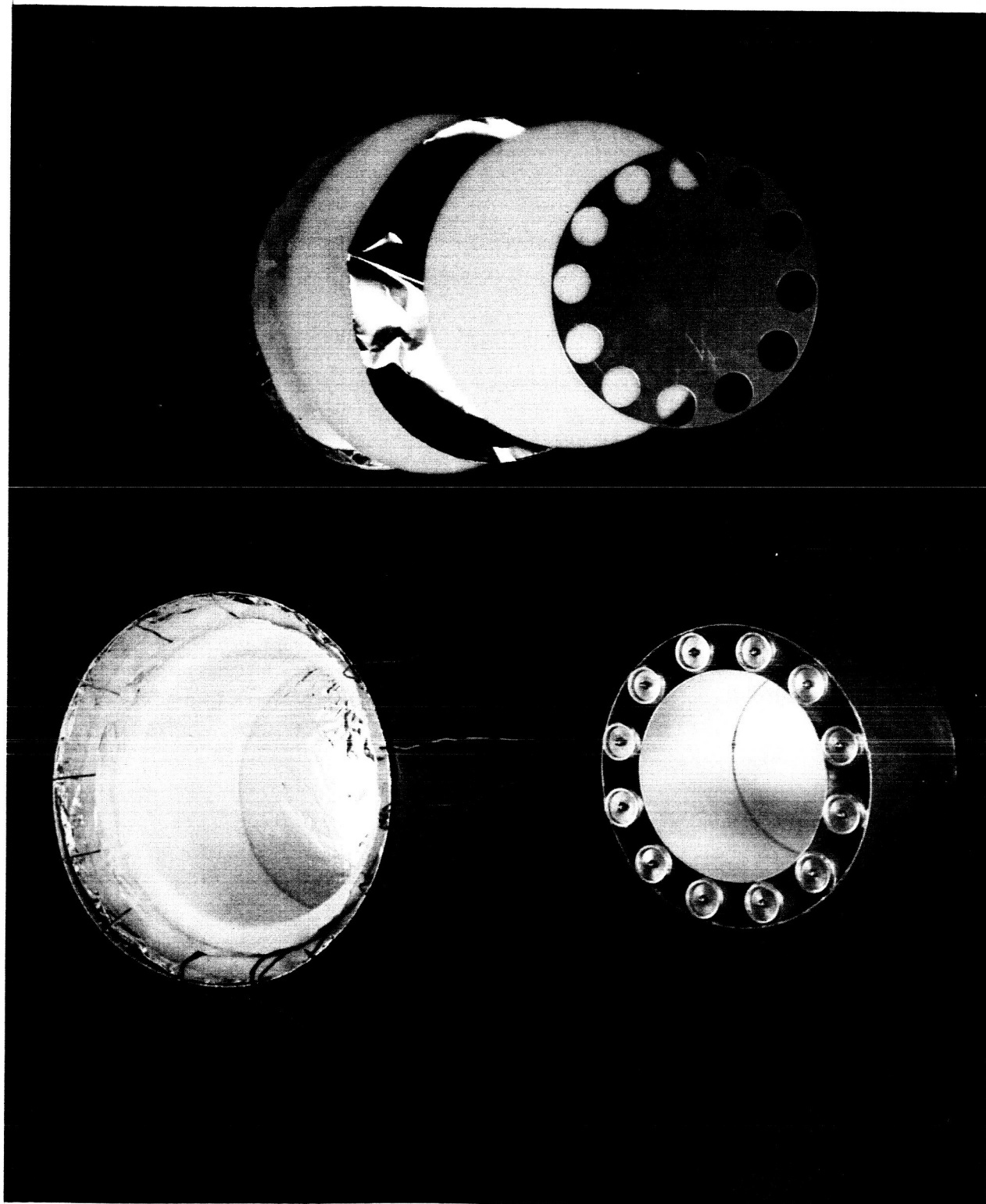SECTION A-A

Figure 25. Cross Section of a Chemical Heater Cartridge

LEAD WIRE

STAINLESS STEEL WASHER
.500" d. X .050TH
.110 CENTER HOLE

CRIMP

FEED THROUGH
(PRESS FIT)

Fe_2O_3/Al
BOOSTER

BRIDGE WIRE (COATED
WITH Zr/BaCrO$_4$ PRIMER)

25 SEC/IN
DELAY LINE
POWDER

1) PACK DELAY RING AT
PRESSURE OF 12,000 PSI

1/8"

3"

.200"

1/8"

3/8"

5/8"

Figure 26. Photograph of Oven

EXTERNAL PORT
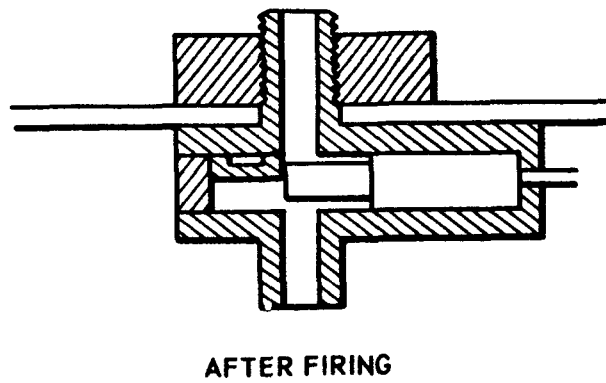
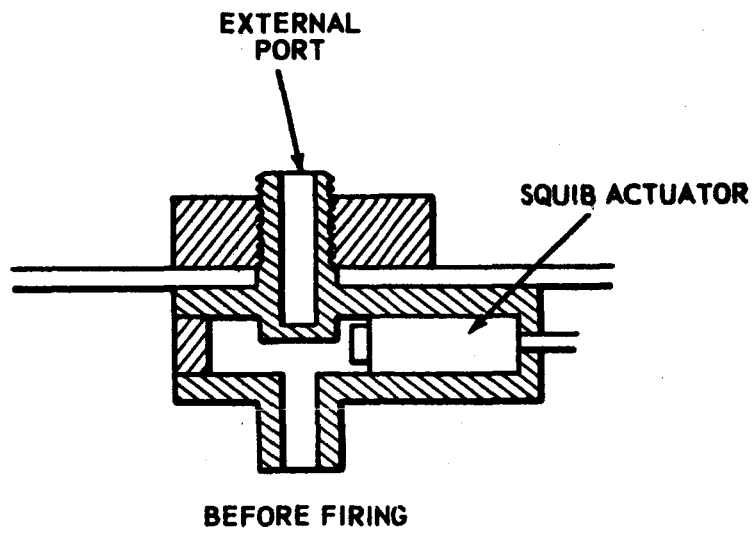SQUIB ACTUATOR

BEFORE FIRING

AFTER FIRING

Figure 27. Inlet or Exhaust Port Concept

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